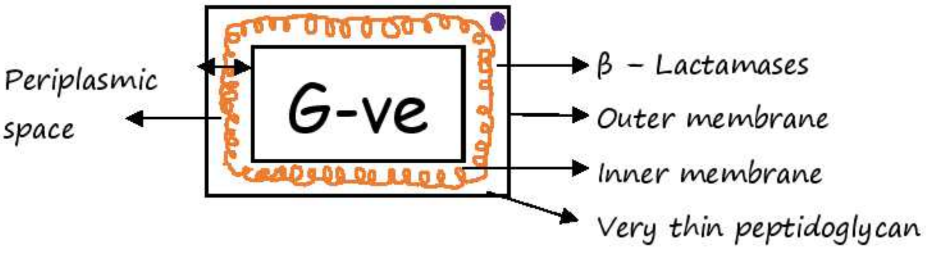
# BACTERIAL CELL ANATOMY/STRUCTURE

## Cell membrane/Plasma membrane/Inner membrane 00:01:40

- It is 5-10 nm thick
- It has respiratory enzymes
- It is Semipermeable/Selectively permeable
- It is a phospholipid bilayer
- Invaginations of cell membrane are called as Mesosomes/Chondroids
- Mesosomes: Are more prominent in gram positive bacteria
- Functions of mesosomes – Respiration
  - Cell division

## MCQ 00:06:32

- Mesosomes are absolutely essential for the life of a bacterium
- In gram negative organisms, there are Inner and outer membranes


Periplasmic space
G-ve
β – Lactamases
Outer membrane
Inner membrane
Very thin peptidoglycan

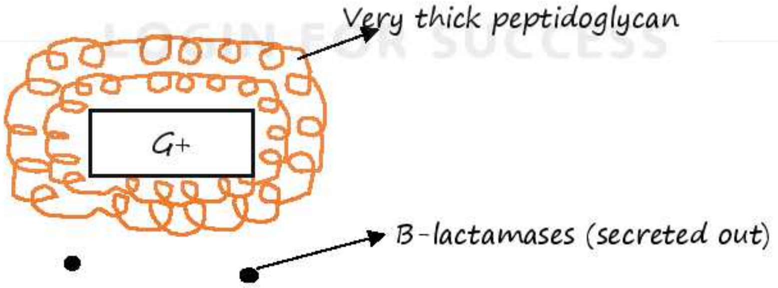
- Outer membrane is a phospholipid bilayer and it has porins (proteins) and endotoxin

## MCQ 00:08:21

- Porins are present in gram negative bacteria
- Outer membrane is present in G- and absent in G+ bacteria
- The space between inner and outer membrane is PERIPLASMIC SPACE which contains very thin PEPTIDOGLYCAN layer
- β – lactamases are also present in the periplasmic space of some G- bacteria. Itbreaks down beta lactam antibiotics

## Gram positive bacteria

- Gram positive bacteria do not have outer membrane
- They have very thick peptidoglycan layer



- β – Lactamases are produced and thrown out or secreted out in the surrounding medium
- CELL ENVELOPE: It refers to all the layers surrounding the bacterial cytoplasm
- Cell envelope is very thick in G+ bacteria

- Cell envelope is more complicated in G- bacteria

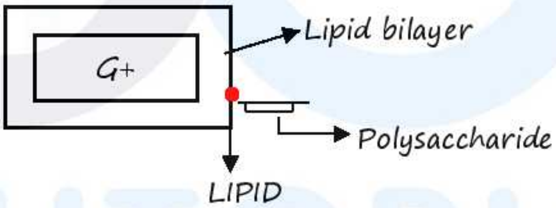
## MCQ 00:13:57

Q-Which kind of bacterial infections are more difficult to treat?

A-Gram negative bacteria (beacause lipid bilayers act as barriers to the entry of several molecules including antibiotics)

## Endotoxin/Lipopolysaccharide (LPS) 00:16:23

- It is present in the outer membrane of gram negative bacteria (Figure 1)


G+
Lipid bilayer
Polysaccharide
LIPID

- It is called as endotoxin because it causes endotoxic shock or gram-negative shock

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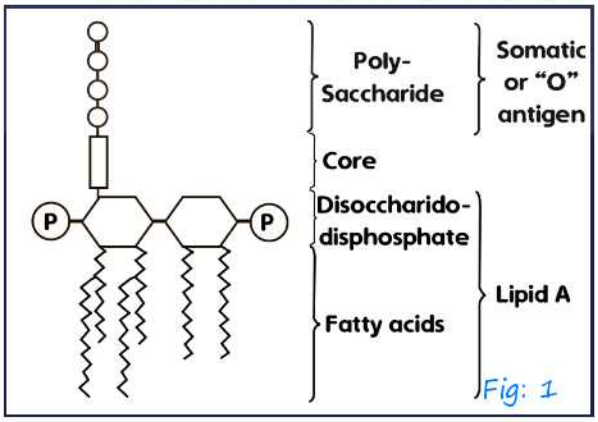
Poly-
Saccharide
Somatic
or "O"
antigen
Core
Disoccharido-
disphosphate
Lipid A
Fatty acids
P
P

Fig: 1

- LPS has got 3-parts – a. Lipid part
  b. Core polysaccharide

c. Outer polysaccharide

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MCQ 00:18:22

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a. Lipid part is Lipid - A

- Lipid-A: It is the toxic moiety responsible for endotoxic shock
- Lipid-A has 2 parts:
  - Disaccharide diphosphate
  - Fatty acids e.g. β – Hydroxy myristic acid

b. Core polysaccharide is made up 3-5 sugars

c. Outer polysaccharide is made up of 25 repeating units of 3-5 sugars

- It is also referred to as somatic or "O" antigen
- Outer polysaccharide varies a lot amongst different bacteria and therefore used for serotyping
- Serotyping is done for classification of organisms below the species level e.g. In S. typhi there are > 200 serotypes

↓

Based on "O" antigen $O_1$ to $O_{200}$

- LPS is called endotoxin in which lipid-A part is similar in structure in all gram-negative bacteria
- Hence the term "Endotoxic shock/Gram negative shock"
- Lipid-A is attached to the core polysaccharide by Ketodeoxy octulunic acid (KDO)

- A gram positive bacterium with endotoxin in its structure is Listeria monocytogenes
- Endotoxin is released on the death of gram-negative bacteria except Neisseria which secretes it while alive
- In Neisseria, the endotoxin is called Lipo-Oligosaccharide (LOS)

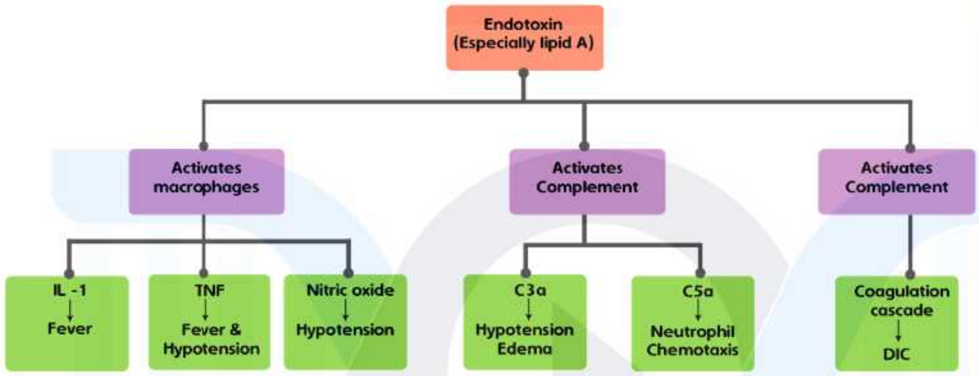
Endotoxin
(Especially lipid A)
Activates macrophages
Activates Complement
Activates Complement
IL -1 → Fever
TNF → Fever & Hypotension
Nitric oxide → Hypotension
C3a → Hypotension Edema
C5a → Neutrophil Chemotaxis
Coagulation cascade → DIC

Fig: 2

## Action of Endotoxin

- Endotoxin (especially lipid A) will activate – Macrophages (M)

  - Complement (C)

  - Tissue factor (T)

- Activation of complement leads to the production of:

  C3a – Hypotension and oedema

  C5a – Causes neutrophil chemotaxis

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- Activation of tissue factor leads to the activation of coagulation cascade which causes Disseminated Intravascular Coagulation (DIC)

LPS/Endotoxin

| **Lipid** | **Polysaccharide** |
|---|---|
| - It is toxic<br>- It is non-immunogenic/non-antigenic | - It is non-toxic<br>- It is immunogenic/antigenic<br>- Outer polysaccharide/O antigen is used for epidemiological studies |

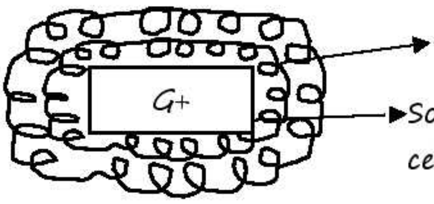
## Adhesins 00:37:18

- Are the structures which helps the bacteria to attach to the host cell

Adhesins

| In gram +ve Bacteria: | In gram –ve bacteria: |
|---|---|
| - Teichoic acid | - Fimbriae/Pili (Singular-pilus) |

- Teichoic acids
  - These are polymers of Ribitol phosphate or Glycerol phosphate
  - They arise from the cell wall
  - In some G+ve bacteria the Glycerol phosphate arises from the lipid part of cell membrane



Teichoic acid (Normally)

Sometimes it arises from lipid part of cell membrane – Lipoteichoic acid

- Lipoteichoic acid has endotoxin-like activity
- Fimbriae/Pili (Singular pilus):

  Are highly antigenic

### Classification of pili/fimbriae

- Mannose sensitive pili:
  They attach to
  mannose sugar
- Mannose resistant:
  They attach to sugars
  other than mannose

## Flagella 00:44:52

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- These are the organs of locomotion in bacteria
- Longer than pili (pili are shorter and straighter than flagella)
- Has 3 parts

  1.Basal body

  2.Hook

  3.Filament
- Made of protein called flagellin (has flagellar or "H" antige
- Flagella is present in some bacteria, not all so H-Antigen can be present in G+ or G- bacteria

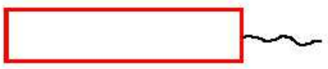
  - O-Antigen is present only in G- bacteria

## MCQ 00:47:42

### Arrangements of flagella

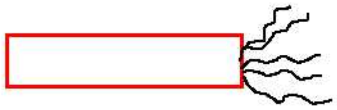
1. Rod Bacillus

Examples



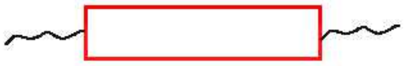
Unipolar/Monotrichous arrangement

- Vibrio cholera (G-)
- Pseudomonas aerqimosa (G-)



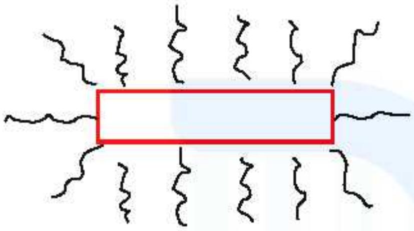
Lophotrichous arrangement

- Helicobacter pylori
- It is rigid Gram -ve spiral bacterium



Amphitrichous arrangement

- Spirillum minus
- Alkaligenes faecalis



Peritrichous arrangement

- E. coli
- Salmonella
- Proteus

↓

- All these belong to the family (G-ve rods) Enterobacteriaceae

Motile: Peritrichous

Non-motile

## MCQ 00:57:44

- Types of motility: DTG

1.Darting/Shooting star motility: Motility which is tough to be traced

- e.g. Vibrio cholerae
- Campylobacter jejuni

2.Tumbling motility: Rolling over on oneself

- e.g. Listeria monocytogenes (G +ve Bacterium)

Motile at 25°c (Room temperature)

Non motile at 37°c

- This is called as Differential motility – Due to expression of gene for flagella at 25°c

3.Gliding motility

- Seen in Mycoplasma
- Mycoplasma do not have flagella
- This is the only motility which is not due to flagella
- Mycoplasma is the smallest medically important bacterium (0.1-0.3µm)
- Only bacterium which does not have a cell wall
- Only bacterium which has sterols in the cell membrane

4.Stately motility: Sluggish motility (BSS)

e.g. Bacillus-G +ve rods

- Clostridium-G +ve rods
- Both these are spore producers too

5.Swarming motility: Swarming is also a growth pattern

e.g. Proteus (G-ve bacterium)

- Clostridium tetani (G+ve bacterium )
- Bacillus cereus (G+ve bacterium)

6.Motility in spirochaetes:

- Cork-screw
- Flexion-extension
- Translatory motion/Spring-like action

## MCQ 01:10:51

**Parasites which are motile:**

1. Giardia lambia/Giardia intestinalis/Giardia duodenalis (Protozoan)
   - It shows falling leaf motility
2. Trichomonas vaginalis (Protozoan): Looks like an inverted pear
   - It shows twitching motility or jerky motility

## MCQ 00:57:44

- Motility is seen by hanging drop preparation under light microscopy
- Indirectly one can visualize the flagella under electron microscope

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## Glycocalyx 01:16:16

- It is the outermost part of bacterial cell envelope
- It is an optional structure
- When it is loosely arranged around bacteria – SLIME
- When it is arranged tightly around bacteria – CAPSULE
- Glycocalyx is made up of polysaccharide

- Capsule is made up of polysaccharide except

Bacillus anthracis (G+)
Yersinia pestis (G-):causes Plague

They have protein capsule

- Bacillus anthracis: It is the largest medically important bacterium, it is non-motile

**Examples of capsulated microbes:**

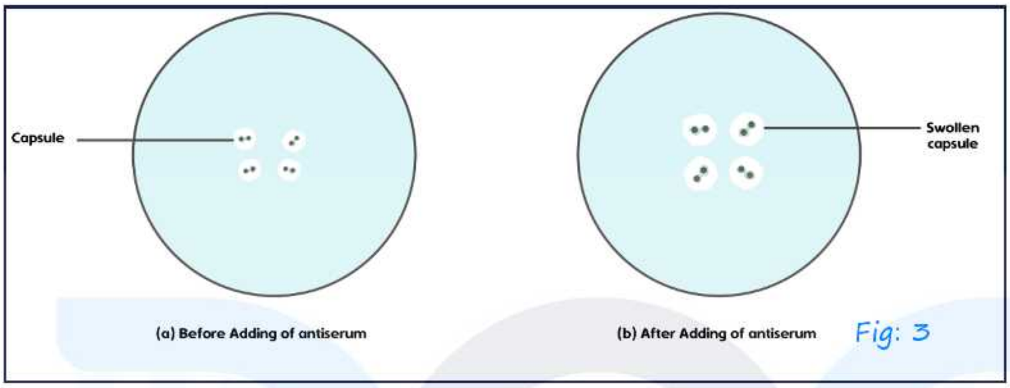
**Mnemonic:**

- Some
    - Streptococcus pneumoniae/Pneumococcus
    - Streptococcus pyogenes
    - Streptococcus agalactiae
- Kreatures → Klebsiella pneumoniae (G-)
- Have → Haemophilus influenzae (G-)
- Pretty → Pseudomonas aeruginosa (G-)
- Nice → Neisseria meningitidis (G – Diplococcus)
- Capsules → Cryptococcus neoformans (Yeast/True yeast) (G +ve)

Histoplasma capsulatum (a fungus) is NOT capsulated

**Capsule can be detected by**

- Negative stains:
  - India Ink
  - Nigrosin
- Quellung reaction/
  Capsular swelling reaction



(a) Before Adding of antiserum

(b) After Adding of antiserum

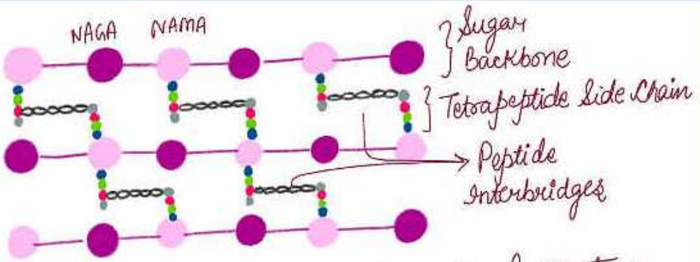
Fig: 3

- Quellung reaction – Pneumococcus (Figure 3):
  If antiserum is added to the sample, antibodies will bind to the capsular antigen of the organism if the organism is present in the sample, hence the capsule appears swollen

## Peptidoglycan 01:33:22

PEPTIDO GLYCAN
↓ ↓
Peptide Sugar → It forms the bulk/weight of the cell wall

- It forms the cell wall of bacteria
- Peptide part contributes to the strength of cell wall
- Glycan part (Sugar): Made up of alternating units of:
  N – acetyl glucosamine (NAGA)
  N – acetyl muramic acid (NAMA)
- NAGA – Is also present in fungal cell wall
- NAMA – Unique/peculiar to bacterial cell wall


NAGA
NAMA
Sugar Backbone
Tetrapeptide Side Chain
Peptide Interbridges

Multilayered, three dimentional structure of Peptidoglycan cell wall.

Fig: 4

- Alternating NAGA and NAMA units
- NAMA is attached to 4-Amino acids: Tetrapeptide side chains
- 3rd amino acid of tetrapeptide of NAMA is attached to the last amino acid of tetrapeptide of next layer of sugar backbone by a PEPTIDE Inter-bridge (Figure 5)

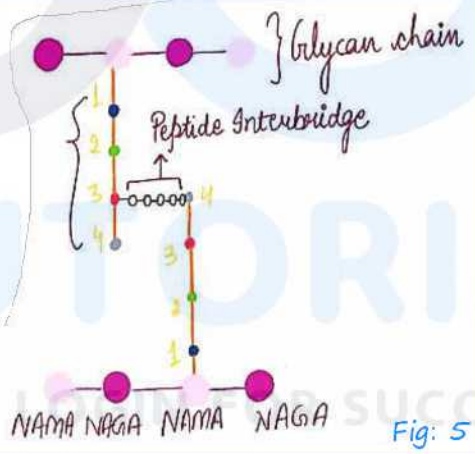

Glycan chain
Peptide Interbridge
1
2
3
4
4
3
2
1
NAMA
NAGA
NAMA
NAGA

Fig: 5

A → Alanine → Laevorotatory (L)

I → Iso-glutamic acid → Dextrorotatory (D)

L → Lysine → Laevorotatory (L)

A → Alanine → Dextrorotatory (D)

- PG cell wall is a highly cross-linked structure
- It is a polymer

## Functions of cell wall 01:42:24

1. Protection against osmotic burst:
   - The interior of a bacterial cell in hyper-osmolar
   - The exterior of a bacterial cell is hypo-osmolar
   - The movement of water is therefore from outside to inside the cell, cell does not burst though but only swells up
   - This is due to the extensively cross-linked cell wall which protects the cell from osmotic burst
   - Mycoplasma: Only bacterium which does not have a cell wall,

     But due to the presence of sterols in its cell membrane, protection against osmotic burst is provided. Lack of cell wall makes it a pleomorphic bacterium
2. Provision of shape and rigidity to the bacterial cell

   Antibiotics acting on bacterial cell wall:
   - Penicillins
   - Cephalosporins
   - Bacitracin
   - Teicoplanin ⎤ Glycopeptide antibiotics
   - Vancomycin ⎦
   - Glycopeptide antibiotics are gigantic molecules which are effective against only G+ve bacteria and not gram-negative bacteria

(Because In G+ve bacteria there is a very thick peptidoglycan layer easily accessible to these gigantic antibiotics compared to G-ve bacteria which have a very thin peptidoglycan layer in the periplasmic space)

## Cell wall deficient forms 01:51:48

1. Protoplasts: Arise from G+ve Bacteria
2. Spheroplasts: Arise from G-ve Bacteria

- They can revert back to cell walled forms

(Because they have some remnants of cell wall precursors)

- NOTE: Mycoplasma is a stable L – form

## Plasmid 01:55:13

- It is extra chromosomal genetic material present in some bacteria
- It is not essential for the life of a bacterium
- Plasmid is present in prokaryotes
- It is an autonomous self-replicating entity
- It is smaller than the bacterial genome
- Bacterial genome ≈ 3000 genes

**Plasmid has 1/10th of bacterial genes (≈ 300 genes)**

## Functions of plasmid

- Pilus
- Light (UV) resistant DNA repair enzymes
- Antibiotic resistance enzymes
- Secreted exotoxins
- Metal reductase enzymes – Inactivate heavy metals like mercury present in Merthiolate
- Nitrogen fixation enzymes
- Degradative enzymes

# TEMPERATURE, OXYGEN, CARBON DIOXIDE, $P^H$ AND SALT REQUIREMENTS OF MICROBES

## Temperature Requirements of Microbes

00:00:10

- Psychrophiles: (Psychro-low, philes-to love)
  - These are the microbes which grow at a low temperature (<20°c)
- Mesophiles:
  - Microbes which grow at 20-40°c
- Thermophiles:
  - Microbes which grow at a high temperature (>50-60°c)
  - Mesophiles are medically important microbes
- Psychrotroph: Microbes which grow at 20-40°c but can also grow at a low temperature
  - E.g., of a psychrotroph: Listeria monocytogenes ($G^+$ Coccobacillus)
  - It grows at a low temperature (≈ 4°c)
- Campylobacter jejuni: Grows at 37°c and 42°c
  - Rigid, G-ve spiral rod

  E.g., of Thermophiles:

  i. Geobacillus stearothermophilus:

  The spores of this bacterium are used as biological indicator for autoclave

  ii. Thermus aquaticus: found in hot water springs, it produces

Taq polymerase which is used in Polymerase Chain Reaction (PCR)

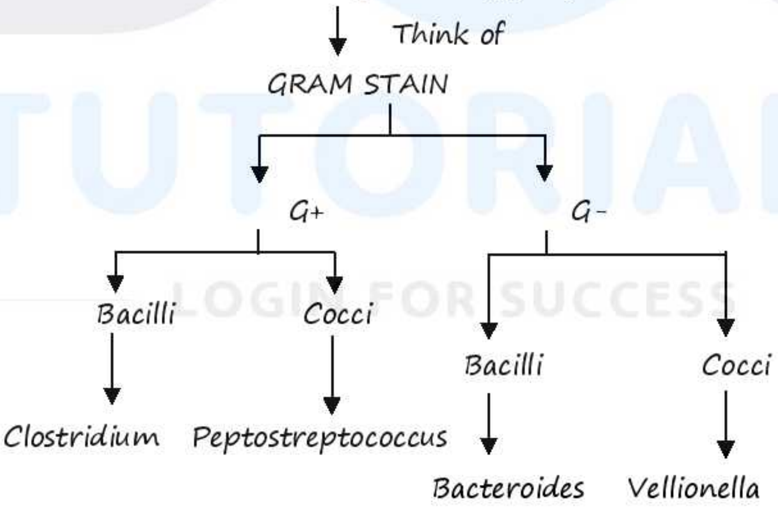
## Oxygen Requirement of Microbes 00:09:36

i. Strict/Obligate aerobes: Need oxygen to grow

- Examples are:

- Nagging: Neisseria
- Pests: Pseudomonas aeruginosa
- Must: Mycobacterium tuberculosis
- Breath: B — Brucella / Bordetella
- Loudly: Legionella

ii. Obligate/Strict Anaerobes: (Hate oxygen)


Think of
GRAM STAIN
G+
G-
Bacilli
Cocci
Bacilli
Cocci
Clostridium
Peptostreptococcus
Bacteroides
Vellionella

iii. Facultative anaerobes: these microbes survive/grow both in the presence or absence of oxygen (Usually they are aerobic)

- E.g: The family Enterobacteriaceae
- Microaerophiles: Microbes which need 5-10% $O_2$ for growth

E.g. *Campylobacter jejuni*

- *Helicobacter pylori*
- Spirochaetes

iv. Aerotolerant anaerobes: Not so strict anaerobes!

- These are the anaerobes which can survive in traces of oxygen

  E.g. *Clostridium perfringens*

  *Clostridium histolyticum*

- Oxygen requirements of bacteria (Figure 1)

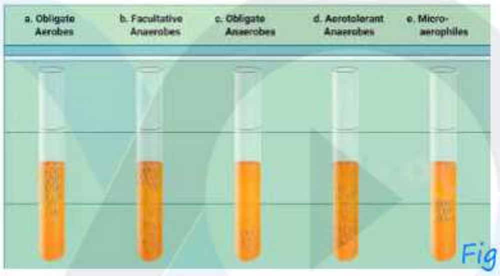

a. Obligate Aerobes
b. Facultative Anaerobes
c. Obligate Anaerobes
d. Aerotolerant Anaerobes
e. Micro-aerophiles

Fig: 1

- McIntosh Filde's jar:

  Used to grow anaerobes

## Carbon dioxide Requirement of Microbes

00:21:30

- Capnophiles: Microbes which need 5-10% of $CO_2$ for optimum growth
- Examples:
  - *Brucella abortus*
  - *Streptococcus pneumoniae*
  - *Campylobacter jejuni*
  - *Neisseria*

- $H_2$
  - Helicobacter pylori
  - Haemophilus influenzae

**Candle jar (Figure 2)**

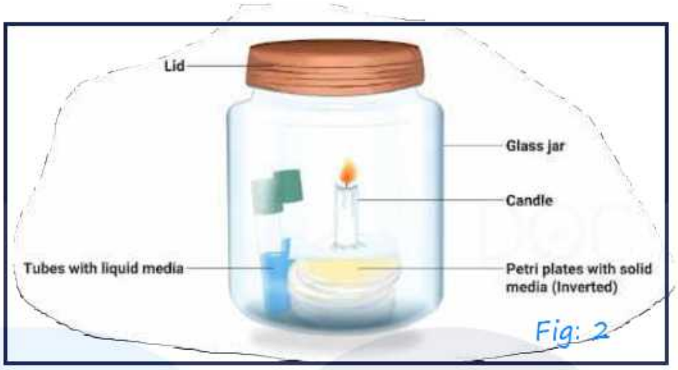

Lid
Glass jar
Candle
Tubes with liquid media
Petri plates with solid media (Inverted)

Fig: 2

- It is used to grow capnophiles

## $P^H$ Requirements of Microbes 00:25:30

- Extremes of $P^H$ is detrimental for microbes
- Human body $P^H$ is 7.4
- Most of the culture media have $P^H$ around 7.4
- Vibrio cholera: Grows at an alkaline $P^H$ (8.2-8.5)

  It is a G-ve curved rod, motile by single/unipolar flagellum

## Salt Requirements of Microbes 00:27:40

- Halophiles (Halo-salt): These are marine organisms

  E.g. Genus Vibrio

  Non – Halophilic Vibrios are:

i. Vibrio cholerae

ii. Vibrio mimicus – Named so because it mimics the non-halophilic property of V. cholerae

(Chief minister → HTN → Less salt)

# BACTERIAL GENETICS PART – I

For you!
I LOVE IT!
Bacteria give the most thoughtful gifts.

Fig: 1

## Horizontal gene exchange 00:02:47

- 3 methods of horizontal gene exchange
  1. Transformation
  2. Conjugation
  3. Transduction

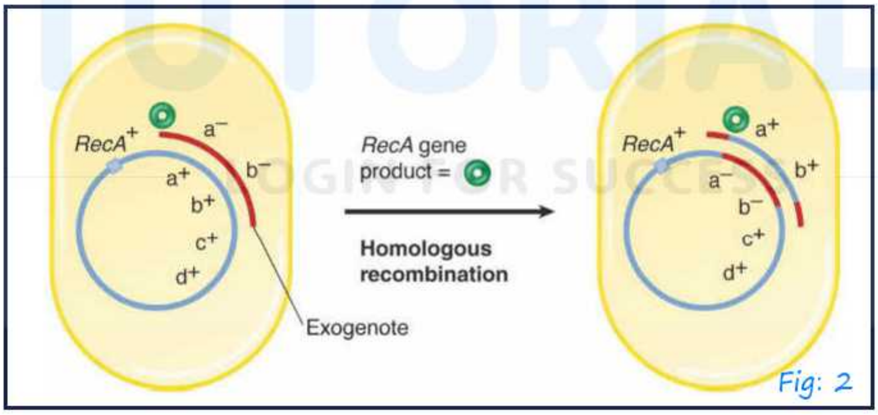

$RecA^+$
$a^-$
$b^-$
$a^+$
$b^+$
$c^+$
$d^+$
RecA gene product =
Homologous recombination
Exogenote
$RecA^+$
$a^+$
$b^+$
$a^-$
$b^-$
$c^+$
$d^+$

Fig: 2

- Genetic exchange in homologous recombination is possible when 2 conditions are met:
  1. Genes have to be homologous
  2. Presence of recombinase enzyme

## Site specific recombination 00:07:20

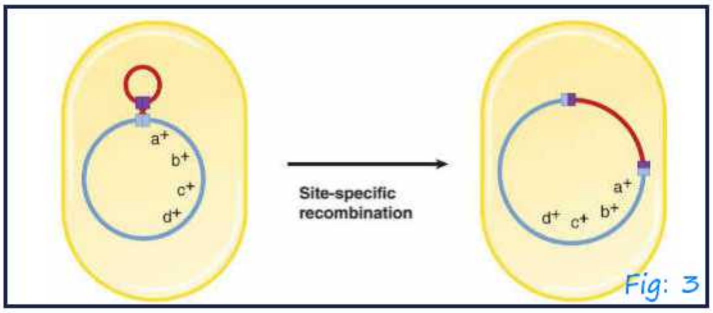

a+
b+
c+
d+
Site-specific recombination
a+
b+
c+
d+

Fig: 3

- Method of stabilizing genetic material
  1. Homologous recombination
     a. Genes are homologous
     b. Recombinase – A enzyme is needed
     c. Gene exchange occurs and not gene addition
  2. Site – Specific recombination
     a. Genes need not be homologous
     b. REC-A enzyme not needed
     c. New genes are added

## Transformation 00:11:55

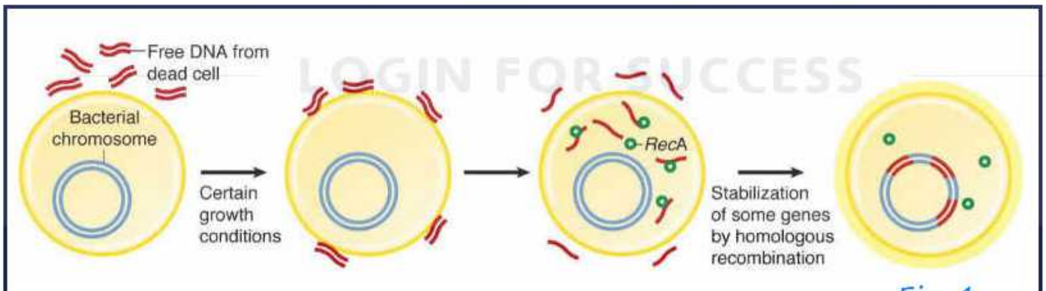

Free DNA from dead cell
Bacterial chromosome
Certain growth conditions
RecA
Stabilization of some genes by homologous recombination

Fig: 4

- Discovered by Griffith in Streptococcus pneumoniae or Pneumococcus
- Also observed in Haemophilus influenzae

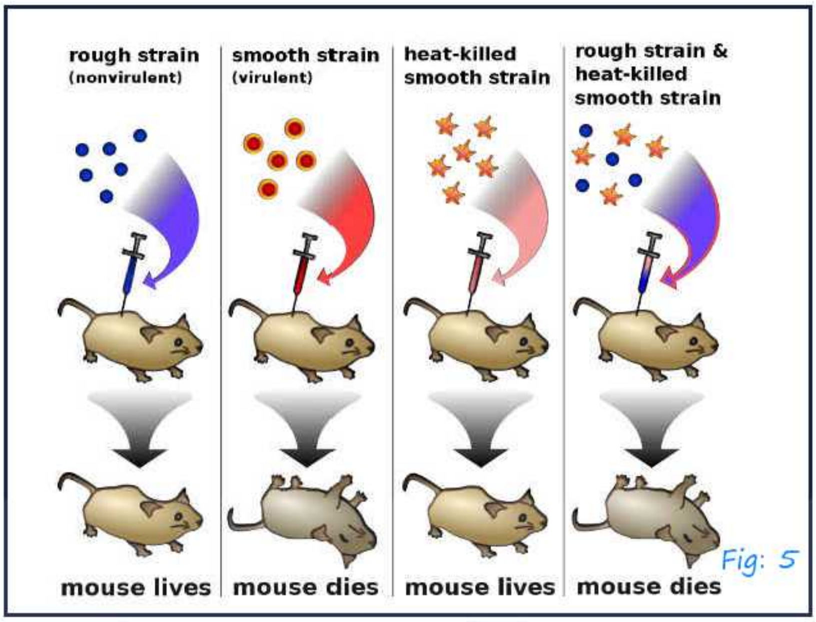
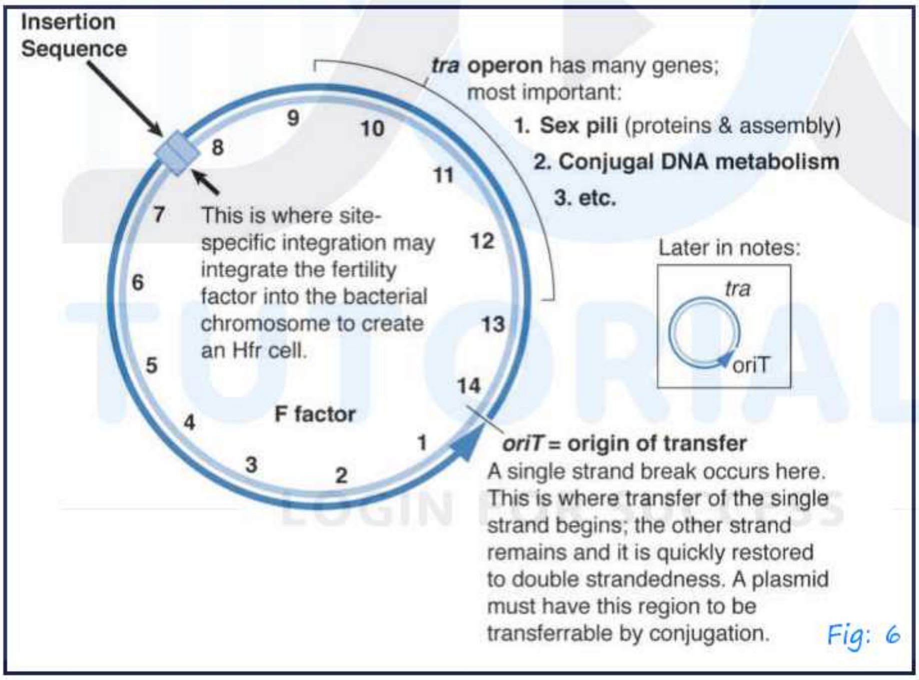

rough strain
(nonvirulent)
smooth strain
(virulent)
heat-killed
smooth strain
rough strain &
heat-killed
smooth strain
mouse lives
mouse dies
mouse lives
mouse dies

Fig: 5

## Conjugation 00:19:40


Insertion
Sequence
1
2
3
4
5
6
7
8
9
10
11
12
13
14
F factor
Later in notes:
tra
oriT

*tra* **operon** has many genes;
most important:
1. **Sex pili** (proteins & assembly)
2. **Conjugal DNA metabolism**
3. **etc.**

This is where site-specific integration may integrate the fertility factor into the bacterial chromosome to create an Hfr cell.

***oriT* = origin of transfer**
A single strand break occurs here. This is where transfer of the single strand begins; the other strand remains and it is quickly restored to double strandedness. A plasmid must have this region to be transferrable by conjugation.

Fig: 6

- Also called F-Factor/Fertility plasmid
- Plasmid –
  - Autonomous self-replicating entities
  - Their replication is independent of replication of bacterial chromosome

## Mating types in bacterial cells: 00:23:59

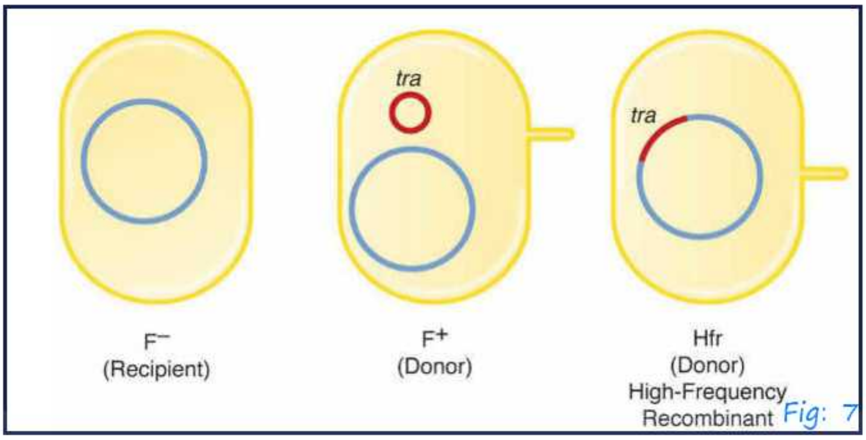

tra
tra
F⁻
(Recipient)
F+
(Donor)
Hfr
(Donor)
High-Frequency
Recombinant

Fig: 7

- F+ By F – Conjugal cross
- HFR By F – Conjugal cross
- HFR – A cell where F-Plasmid integrated with bacterial chromosome

# BACTERIAL GENETICS PART - II

## Transduction 00:00:17

- Bacteriophage – A virus which attacks bacterium

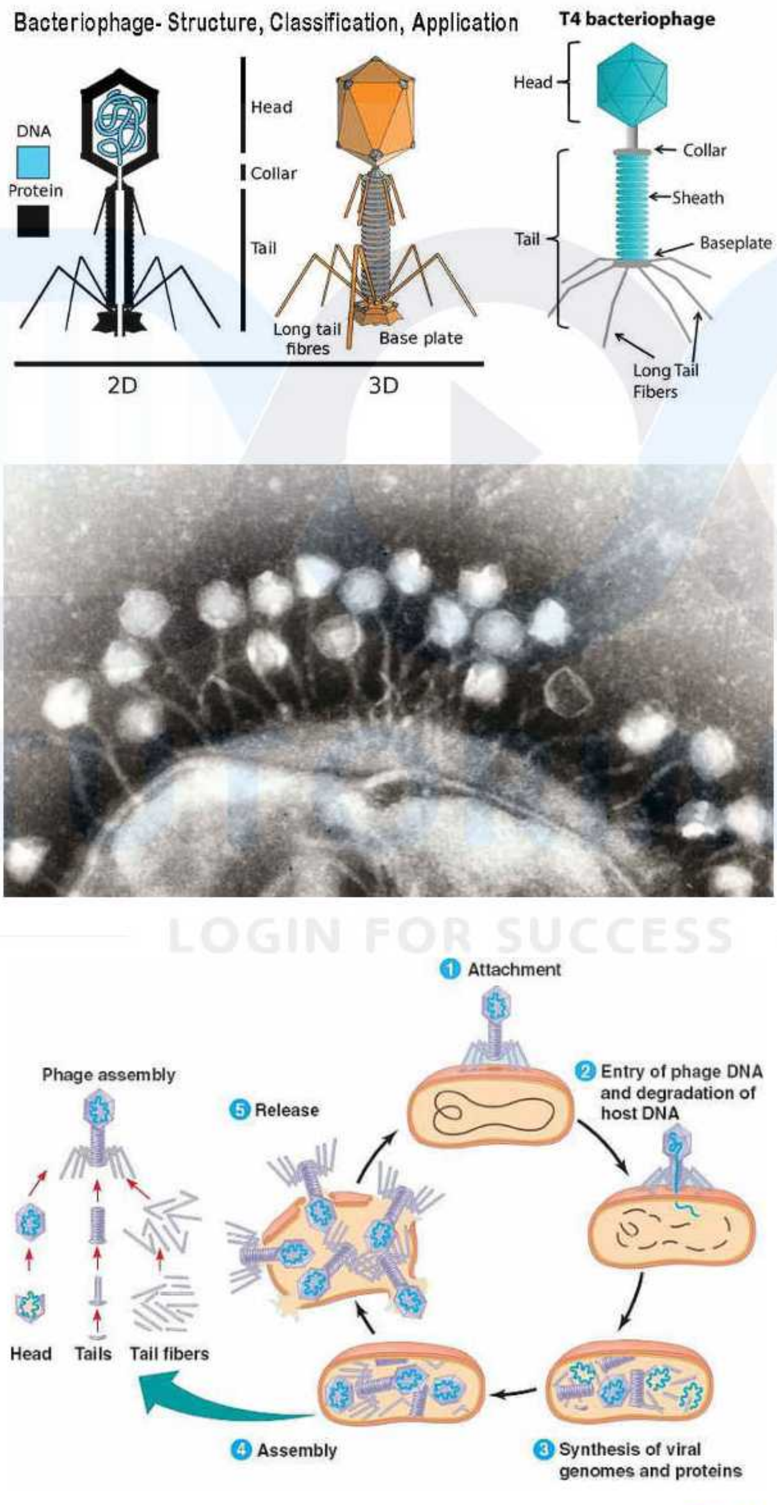
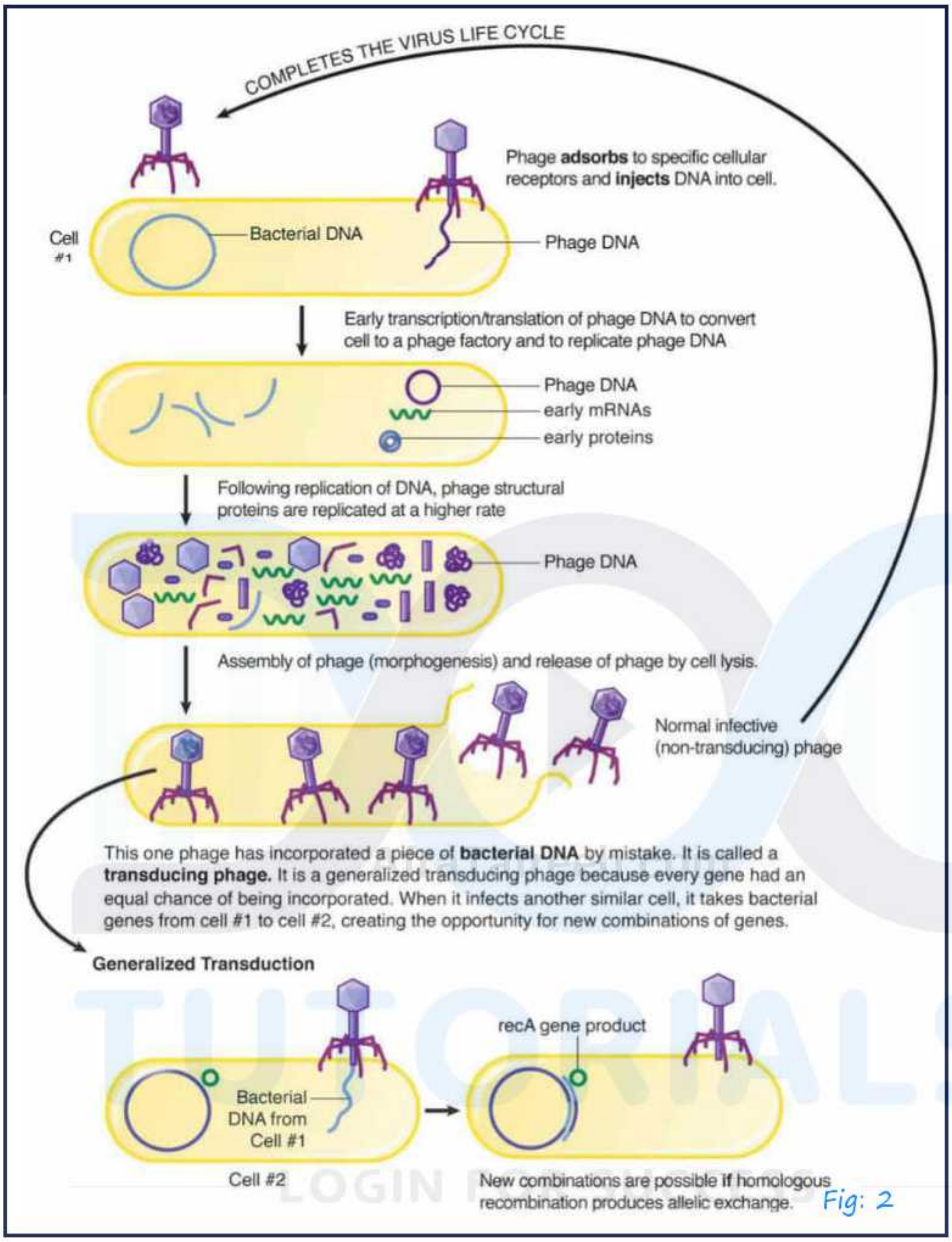

Bacteriophage- Structure, Classification, Application
DNA
Protein
Head
Collar
Tail
Long tail fibres
Base plate
2D
3D
T4 bacteriophage
Head
Collar
Sheath
Tail
Baseplate
Long Tail Fibers
1 Attachment
2 Entry of phage DNA and degradation of host DNA
3 Synthesis of viral genomes and proteins
4 Assembly
5 Release
Phage assembly
Head
Tails
Tail fibers

Fig: 1

# Generalized Transduction


COMPLETES THE VIRUS LIFE CYCLE
Cell
#1
Bacterial DNA
Phage DNA
Phage DNA
early mRNAs
early proteins
Phage DNA
Normal infective
(non-transducing) phage
Generalized Transduction
recA gene product
Bacterial
DNA from
Cell #1
Cell #2

Phage **adsorbs** to specific cellular receptors and **injects** DNA into cell.

Early transcription/translation of phage DNA to convert cell to a phage factory and to replicate phage DNA

Following replication of DNA, phage structural proteins are replicated at a higher rate

Assembly of phage (morphogenesis) and release of phage by cell lysis.

This one phage has incorporated a piece of **bacterial DNA** by mistake. It is called a **transducing phage.** It is a generalized transducing phage because every gene had an equal chance of being incorporated. When it infects another similar cell, it takes bacterial genes from cell #1 to cell #2, creating the opportunity for new combinations of genes.

New combinations are possible **if** homologous recombination produces allelic exchange.

Fig: 2

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## Lysogeny 00:13:25

- Phenomenon well studied in Escherichia coli.

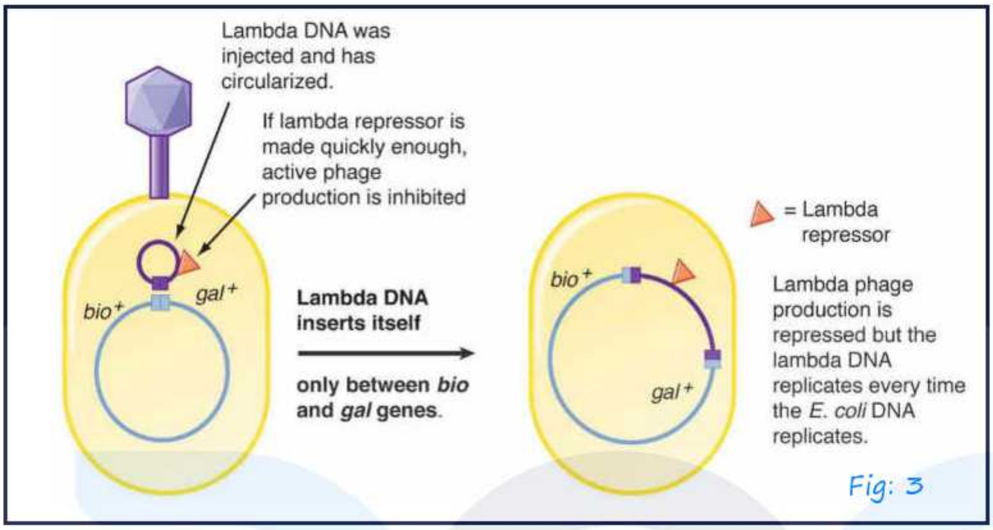
Lambda DNA was injected and has circularized.
If lambda repressor is made quickly enough, active phage production is inhibited
bio+
gal+
Lambda DNA inserts itself
only between bio and gal genes.
bio+
gal+
= Lambda repressor
Lambda phage production is repressed but the lambda DNA replicates every time the E. coli DNA replicates.

Fig: 3

## Normal excision of a lysogenic phase 00:16:58

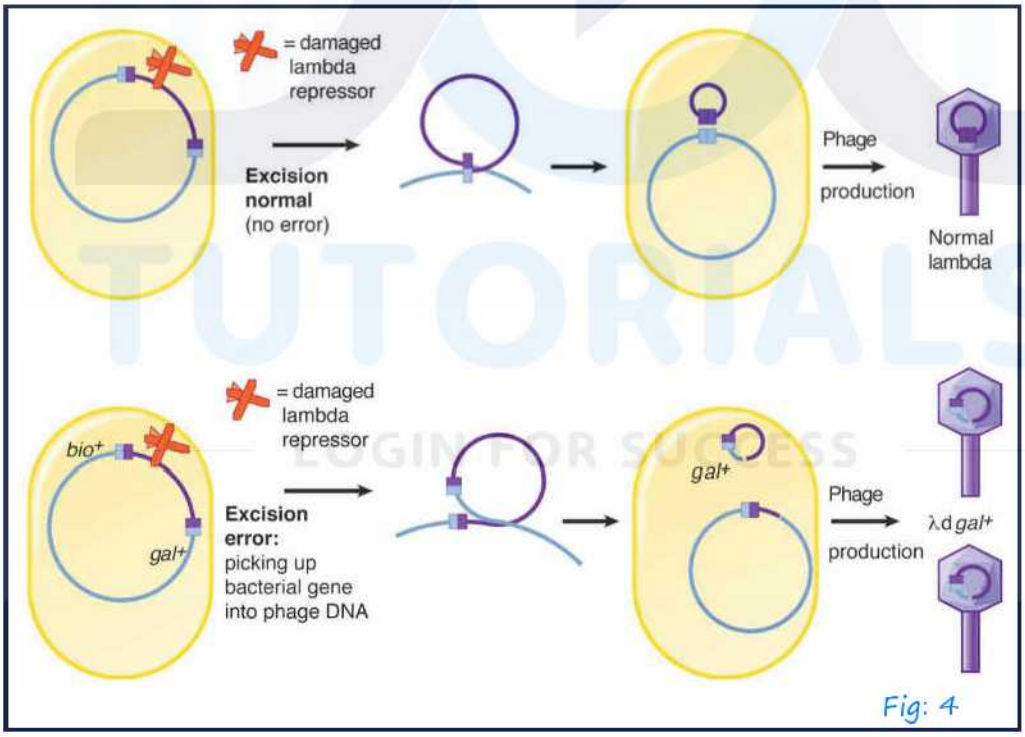
= damaged lambda repressor
Excision normal (no error)
Phage production
Normal lambda
= damaged lambda repressor
bio+
gal+
Excision error: picking up bacterial gene into phage DNA
gal+
Phage production
λd gal+

Fig: 4

## Specialized Transduction

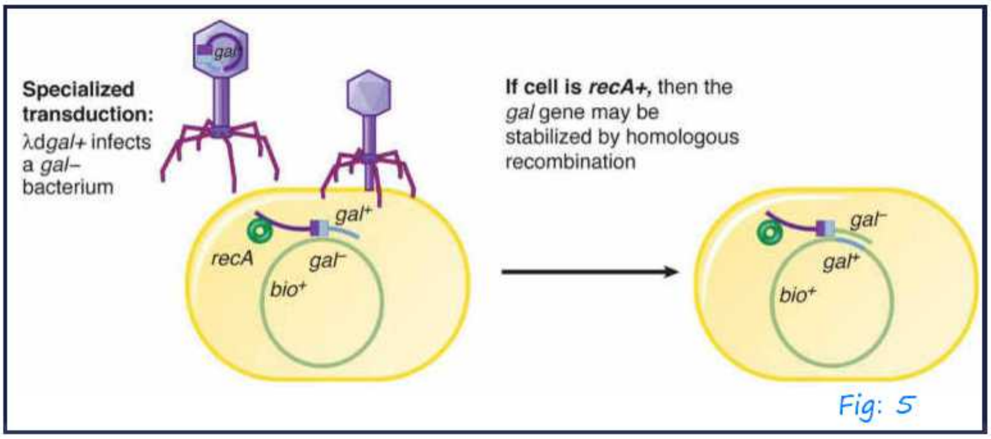
Specialized transduction: λdgal+ infects a gal– bacterium
gal+
gal+
recA
gal–
bio+
If cell is recA+, then the gal gene may be stabilized by homologous recombination
gal–
gal+
bio+

Fig: 5

## Comparison of Transformation, Conjugation and Transduction

00:22:19

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| Requirement | Transformation | Conjugation | Transduction |
|---|---|---|---|
| Is cell-to cell contact required? | No | Yes | No |
| Does it require an antecedent phage infection? | No | No | Yes |
| Is competency required? | Yes | No | No |
| Is naked (free) DNA involved? | Yes | No | No |
| Is recombination required to stabilize new genes? | Yes | No for $F^+$ x $F^-$<br>Yes for Hfr x $F^-$ | Yes |

## Comparison of Generalized and Specialized Transduction 00:24:00

| | Generalized | Specialized |
|---|---|---|
| Mechanism | Error in assembly | Error of excision<br>Requires stable insertion of prophage DNA (lysogeny) |
| What genes may be transferred? | Any | Only genes next to the insertion site |

# INTRODUCTION AND PHYSICAL METHODS – II

## Radiation 00:00:14

**Radiation types**

**Ionizing Radiation**

- X – rays
- Cosmic rays
- Gamma rays (Source – Cobalt-60)

**Non-Ionizing Radiation**

- UV rays
- They have poor penetrating power
- Can cause disinfection and not sterilization

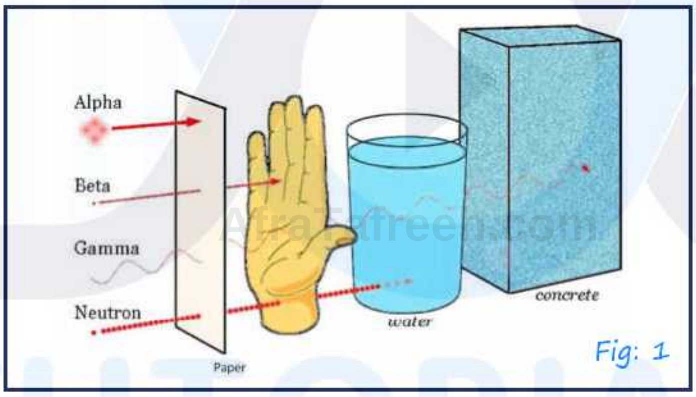

Fig: 1

- Gamma rays have very high penetrating power (Figure 1)

## Uses of Gamma Rays:

- They are used for disinfection and sterilization of disposable plastics
- Like:
  - Plastic syringes
  - Infusion sets
  - Catheters etc.

Also, for disinfection and sterilization of:

- Adhesive dressings
- Bone and tissue grafts
- Catgut sutures
- Antibiotic solutions
- Food (In some countries)
- Mechanism of action of gamma rays: They cause DNA damage
- Biological indicator: Bacillus pumilus

## UV Rays 00:04:36

- Poor penetrating power
- Bring about only disinfection, not sterilization

### Mechanism of action of UV rays

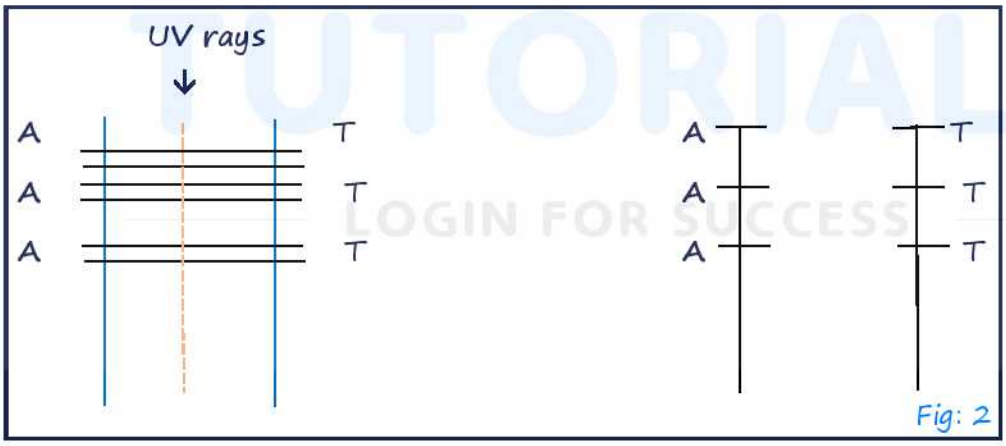

Fig: 2

- Breakage of Hydrogen bonds leading to formation of Thymidine/Pyrimidine dimers (Figure 2)
- At a wave length of 250-260nm, the best antimicrobial activity has been observed because of maximum absorption of UV rays by DNA bases

Uses of UV rays:

- Disinfection of:
  - Wounds
  - Biosafety cabinets
  - Labs
  - Wards
  - OTs
  - Enclosed areas like entry-ways
  - Laminar flow hoods

## Filtration 00:08:38

- Filtration:

1. Done for sterilisation of heat-labile fluids
2. Filtration is also used for separation of bacteriophages and toxins from bacteria
3. To obtain bacteria-free filtrates of clinical samples for viral isolation
4. Purification of water

Types of filters:

## Depth filter 00:11:19

- These filters retain particles throughout their depth before becoming clogged
- Advantages: Cheap, Flow rate of fluids is very high

- Uses: Industrial applications like filtration of food, beverages, chemicals

## Types of depth filters:

**Candle filters**

- **Diatomaceous earth filters**
  - ↓ Berkefeld
- **Ungalzed porcelain filters**
  - ↓ Chamberland

**Asbestos filters**

- **Seitz**
- **Sterimat filters**

- Not used now because of carcinogenic nature
- They cause Mesothelioma of pleura

**Sintered-glass filters:**

- Not used now because they are brittle
- They are made up of crushed glass particles

## Membrane filters 00:16:04

- Membrane filters are most commonly used filters (Figure 3)

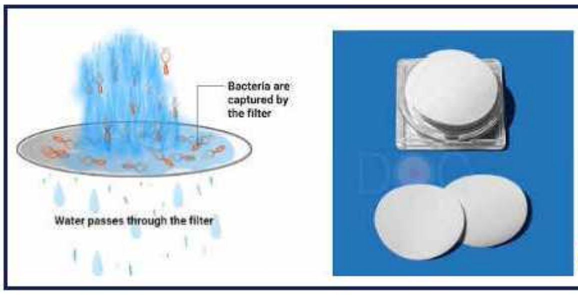
Bacteria are captured by the filter
Water passes through the filter

Fig: 3

- They are made up of Cellulose acetate, Cellulose nitrate, Polycarbonate, Polyvinylidene fluoride

**Pore sizes**

| 0.22µm | 0.45µm | 0.8µm |
|---|---|---|
| • Best<br>• Used most commonly | • Used to remove coliform bacteria in water microbiology | • Used to obtain bacteria-free air in clean rooms |

**Biological Indicators for Membrane filters:**

- *Brevundimonas diminuta*
- *Serratia marcescens*

## Filtration of air 00:20:30

- Ultra-clear air is needed in:
  - OTs
  - ICUs
  - Transplantation units

### Methods of filtration of air:

**1. Surgical Mask:**

- Allows air to pass through but not bacteria

| 2. HEPA filters | 3. ULPA filters |
|---|---|
| High<br>Efficacy<br>Particulate<br>Air<br>Removes 99.97% of particles of ≥ 0.3μm size | Ultra<br>Low<br>Particular<br>Air<br>Removes 99.999% of particles of ≥ 0.12μm |

# CHEMICAL METHODS OF STERILISATION AND DISINFECTION

## Chemical Methods of Sterilisation and Disinfection

00:00:24

**Phenol:**

1. First chemical agent used in medical practice by Sir Joseph Lister
2. Also known as carbolic acid
3. Mechanism of action:
    a. Damages cell membrane i.e. cell lysis causing release of cellular contents
    b. Denaturation of proteins
4. Good action on: (GGM)
    - G +ve Bacteria
    - G –ve Bacteria
    - Mycobacterium tuberculosis
        - Therefore, phenol is the disinfectant of choice for sputum
5. Poor action on
    - Spores
    - Viruses

- Sir Joseph lister: Father of Antiseptic surgery
- Phenol is not a sterilant

## Phenolics:

- Derivatives of phenol

**Phenolics**

| Disinfectants | Antiseptics |
|---|---|
| 1. Lysol | 1. Chlorxylenol (active ingredient in Dettol) |
| 2. Cresol | 2. Hexachlorophene<br>- Causes brain damage<br>- Not used commonly except for Staphylococcal outbreak |
| 3. Xylenol | |
| 4. Ortho-phenyl-phenol | |
| • Widely used in labs as disinfectants | |

- Activity of phenol decreases in the presence of organic matter

## Chlorhexidine Gluconate (CHG) 00:10:58

- It is biguanide
- It is a widely used antiseptic agent in
  - Hand-washes
  - Hand rubs
  - Mouth wash
  - Body wash

- 2-4% CHG: Active against spores of Clostridium difficile
- CHG is:
  - Bactericidal
  - Mycobacteriostatic
  - Sporostatic
- Variable activity on:
  - Yeasts
  - Viruses
  - Protozoa

## Halogens 00:13:28

- Iodine and chorine
- They have antimicrobial effect
- Sodium salts of iodine and chlorine are very commonly used for antisepsis and disinfection

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### Chlorinated compounds:

- Corrosive → therefore not used as antiseptics
  - Used for: Disinfection and not sterilisation
- Examples:

1.Sodium hypochlorite (Household bleach, 5.25%)
2.Calcium hypochlorite (Bleaching powder)
3.Chlorine gas

## Mechanism of action:

- Very good oxidising agents, all release hypochlorous acid/HOCl:

  Oxidation of cellular material and destruction of vegetative bacteria and fungi
- Good action on
  - G +ve Bacteria
  - G –ve Bacteria
  - Viruses

- Sodium hypochlorite is the agent of choice to manage Blood-spill → 1% Sodium hypochlorite is used

- Poor action on:
  - Spores
  - Mycobacterium tuberculosis
- Activity of chlorine **decreases in the presence of organic matter**
- Sodium hypochlorite is:
  - Unstable
  - Disintegrates easily, therefore fresh solution is to be made daily

Chlorine + organic compounds $\xrightarrow[\text{Form}]{\text{To}}$ Trihalomethane (Carcinogenic)

- Activity of Chlorine decreases in the presence of organic matter

## Iodinated compounds:

- Very good **antiseptics**
- Mechanism of action:

  Very good oxidising agents and they iodinate cell proteins
- Effective against most microbes **but not spores**
- Tincture iodine: Preparation of 2% iodine in water-ethanol-solution of potassium iodide
- Side-effects of tincture iodine:
  - Discolouration of skin
  - Allergic reactions
- Not used for disinfection (because of discolouration)
- Iodophors: Compounds made by complexing iodine with organic carriers. Eg: Povidone to form Povidone iodine (Betadine)
- Advantages:
  - No skin allergy
  - No skin discolouration (since, iodine is released slowly from iodophors)
- Uses: Commonly used as pre-operative antiseptics
- Brands:
  - Betadine
  - Wescodine

## Alcohols 00:29:31

1.Used for disinfection and antisepsis
  - But not sterilisation

2.Do not kill spores and kill only enveloped viruses

3.Mechanism of action:
  a. Denaturation of proteins
  b. Possibly act by dissolving membrane lipids

4.Concentration used:
  - 70-80%

5.Types used
  - Ethyl alcohol
  - Isopropyl alcohol

- Note: 100% alcohol (absolute alcohol) is NOT used since denaturation of proteins occurs only in the presence of some water (Therefore 70-80% alcohol used)

6.Isopropyl alcohol:
  - Better than ethyl alcohol because it has:
  - Better penetration
  - Better spreading action
  - Less volatile
  - More bactericidal activity

7.Ethyl alcohol: used as surgical spirit (70%)

8.Isopropyl alcohol is used to disinfect:

  - Clinical thermometers
  - Small surgical instruments
    - This is done by immersing them for 5-10 minutes

## Aldehydes 00:37:02

- Aldehydes – Mechanism of action:
  - Alkylation of
    - i. Nucleic acid
    - ii. Proteins
- Aldehydes are corrosive and toxic, therefore not used for antisepsis
- They are used for disinfection and sterilisation

**Examples:**

1. Formaldehyde:
   - Restricted use, since it is carcinogenic
   - Most commonly used agent is formation/ formol (40% formaldehyde)
   - Uses of formalin:
     - To prepare toxoids form toxins
     - To preserve anatomical specimens

> **It was earlier used for fumigation of operation theaters (now banned)**

   - Side effects:
     - Highly toxic and irritant when inhaled

  - Corrosive and carcinogenic

2.Glutaraldehyde

- G-good: because it is less:
  - Toxic
  - Irritant
  - Corrosive
- Not carcinogenic

**2% solution (Cidex) – is used for disinfection (20 minutes) and sterilization (12 hours) of fiberoptic scopes**

- Disadvantages of CIDEX (Glutaraldehyde):
  - It has to be activated before use by alkalinization.
  - Has short shelf life (≈14days)

3.Ortho-ph-thalaldehyde

- 0.55% solution is used for disinfection and sterilisation of fiberoptic scopes
- Better than glutaraldehyde because:
  - i. Need not be activated before use
  - ii. It has longer shelf life
  - iii. Better odour
  - iv. Low vapour density
  - v. Better mycobactericidal activity

## Ethylene oxide/Eto 00:52:18

- It is a gaseous chemical sterilant
- Mechanism of action: DNA damage
- It is highly:
  - Irritant
  - Inflammable
  - Explosive
  - Carcinogenic
- It has very good penetrating power, rapidly penetrates packing material
- Uses:
  - Used for disinfection and sterilisation of heat-sensitive items. Example- Disposable plastics (plastic syringes, plastic petri dishes)

    Heart-lung machines

    Respirators

    Dental equipments

    Sutures

    Catheters

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- **For catgut sutures – we use gamma rays (I.R)**
- **For other sutures we use ethylene oxide**

- 10-20% of ethylene oxide is mixed with an inert gas
  - Eg: $CO_2$ or dichloro-difluoro-methane
- At a concentration of 700 mg/L and 40-80% relative humidity, Eto achieves sterilisation at 55°c in 1 hour

- At 38°c in 4-5 hours

- Biological indicator: Bacillus globigi

## Surface active agents/Surfactants 00:58:21

- They decrease the surface tension between two liquids or between a liquid and a solid
- Are good
  - Detergents
  - Emulsifiers
  - Wetting agents
- They are amphipathic (Both hydrophilic and hydrophobic groups)

1. Cationic surfactants:
   - They have a net positive charge
   - Good microbial activity: kill G +ve Bacteria better than G –ve Bacteria
   - Do not kill spores, Mycobacterium tuberculosis
   - Kill only enveloped viruses and not non-enveloped viruses
   - Uses: Used as Disinfectants → utensils, instruments
     - As antiseptics
   - Mechanism of action: Damage to cell membrane
   - Most commonly used cationic surfactants are:

Quaternary ammonium compounds

Cetrimide (cetavlon)

Benzalkonium chloride

- Active ingredients of savlon:

Savlon: It has 2 active ingredients:
i. Cetrimide, ii. Chlorhexidine

2.Anionic surfactants: Have a net negative charge

- Are more active at acidic pH
- Very good detergent activity
- Example: Common soaps

3.Amphoteric surfactants:

- Have equal positive and negative charges; net charge is zero
- Have antimicrobial activity of cationic agents and detergent activity of anionic agents
- Activity of these compounds decreases in the presence of organic matter
- Are active at a wide range of pH
- Example: TEGO compounds: Widely used antiseptics in dental practice
  - Side effects of TEGO compounds: Allergic reactions
  - Active ingredient is: DDAG (Dodecyl-di-aminoethyl glycine)

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## Oxidising agents 01:07:37

### Oxidising agents

| Hydrogen peroxide ($H_2O_2$) | Per-acetic acid |
|---|---|
| Most common concentration: 3-6%<br>But 1% concentration is used for catalase-enzyme producing microbes | More powerful/ better oxidising agents<br>It is sporicidal at a concentration of <1% even at low temperature |
| Uses | |
| 1. Used in plasma sterilisation (alone or along with per-acetic acid) | 1. Along with $H_2O_2$, it is used for plasma sterilisation |
| 2. Used for disinfection of ventilators, tonometer biprisms, soft contact less | 2. Used for disinfection of dialysis<br>3. Used for sterilisation of endoscopes |

$H_2O_2$ is –

- Environmentally safe
- Non-toxic for humans (neither mutagenic nor carcinogenic)

- Also, it does not coagulate blood or fix tissue to surfaces

## Plasma sterilisation 01:12:11

- "Plasma" → 4th state of matter; which contains ions, electrons, photons and neutral uncharged particles, free radicals
- All of these are microbicidal as well as sporicidal
- Plasma is created in an instrument called plasma steriliser

**Brand name: Sterrad, Plazlyte**

- Created from $H_2O_2$ or a combination of $H_2O_2$ and per-acetic acid
- Whole cycle of sterilisation takes 1 hour
- Uses: Sterilisation of surgical instruments
  Sterilisation of items which can't tolerate high temperature and humidity of autoclaves, for example:
  - Plastic some
  - Electric devices
  - Corrosive susceptible metal alloys
- A low temperature is maintained (37-44°c)
- Biological indicators: Spores of Geobacillus stearothermophilus

## Testing of disinfectants 01:17:03

### 1.Phenol coefficient test/Rideal Walker test:

$$\text{Phenol Coefficient (PC)} = \frac{\text{Highest dilution of test disinfectant which kills Salmonella typhi in a given time}}{\text{Highest dilution of phenol which kills Salmonella typhi in the same time}}$$

- If PC >1, the test disinfectant is superior to phenol

**Disadvantages of PC test:**

- Only phenolic compounds can be tested
- This test does not assess the activity of the test disinfectant in the presence of organic matter

### 2.Chick Martin test:

- It is a modification of phenol coefficient test
- This test assesses the activity of test disinfectant in the presence of organic matter (Dried faeces or yeast extract)

### 3.Capacity test/ Kelsey-Sykes test:

- Assesses the activity of test disinfectant when microbial load is increasing

### 4.In-use test/ Kelsey Maurer test:

- Done in hospitals (once in every 3 months)
- This test assesses whether an actively being used disinfectant is microbiologically contaminated

## Spaulding's classification of medial devices:

| Medical device | Definition | Examples | Recommended sterilisation/ disinfection |
|---|---|---|---|
| Critical device | Enter a normally sterile site | Surgical instruments, cardiac & urinary catheters, implants, eye and dental instruments | Heat-based sterilisation chemical sterilant or high-level disinfectant |
| Semi-critical device | Comes in contact with mucus membranes or minor skin breaches | Respiratory therapy equipment, anesthesia equipment, endoscopes, laryngoscope, rectal/vaginal/ oesophageal probes | High-level disinfection |
| Non-critical devices | Comes in contact with intact skin | Blood pressure cuff, electrocardiography electrodes, bedpans, crutches, stethoscope, thermometer | Intermediate-level or low-level disinfection |
| Non-critical environmental surfaces | Less direct contact with the patient | Surfaces of medical equipment, examination table, computers | Low-level disinfectant |

# INNATE & ACQUIRED IMMUNITY PART - III

## Macrophages 00:00:42

- Monocytes are present in blood
- Macrophages are present in tissues
- They are very good phagocytic cells
- They kill pathogens by oxidative mechanism and non-oxidative mechanism
- They have receptors for Fc part of IgG
- Macrophages are long lived cells

### Examples of macrophages in tissues:

1. Lungs– Alveolar macrophages or Dust cells
2. Liver – Kupffer cells
3. Bones – Osteoclasts
4. Skin – Langerhans cells
5. Kidneys – Mesangial cells
    - Macrophages have a protein on their surface known as CD14

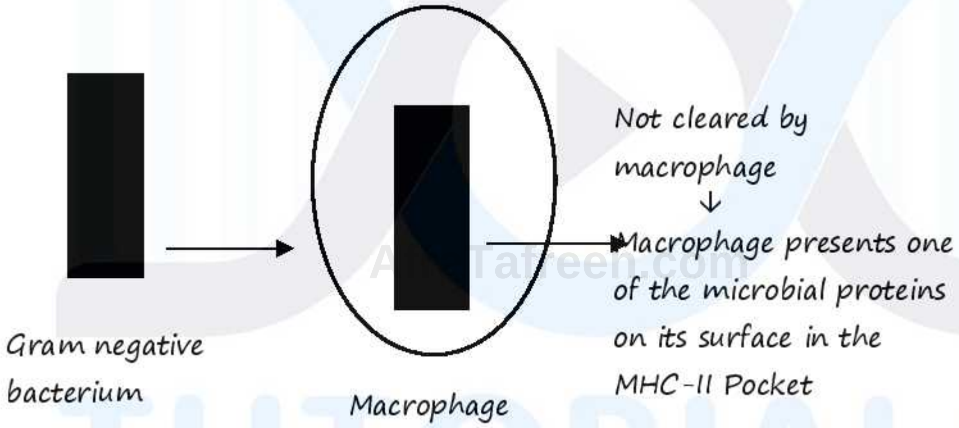
### CD: Cluster of differentiation

- (≈ 350 molecules)

### CGD (Chronic Granulomatous Disease):

- In the setting of an infection neutrophils may not clear the microbe efficiently and they die in a few days

- Later blood monocytes enter the tissues & transform into macrophages
- Macrophages also cannot clear the microbe because of defective NADPH-Oxidase gene
- But unlike neutrophils, macrophages do not die at the site of infection, they persist & form granulomas
- Granulomas: They are produced in response to intracellular organisms
- Macrophages are Antigen Presenting Cells (APCs)


Gram negative bacterium
Macrophage
Not cleared by macrophage
↓
Macrophage presents one of the microbial proteins on its surface in the MHC-II Pocket

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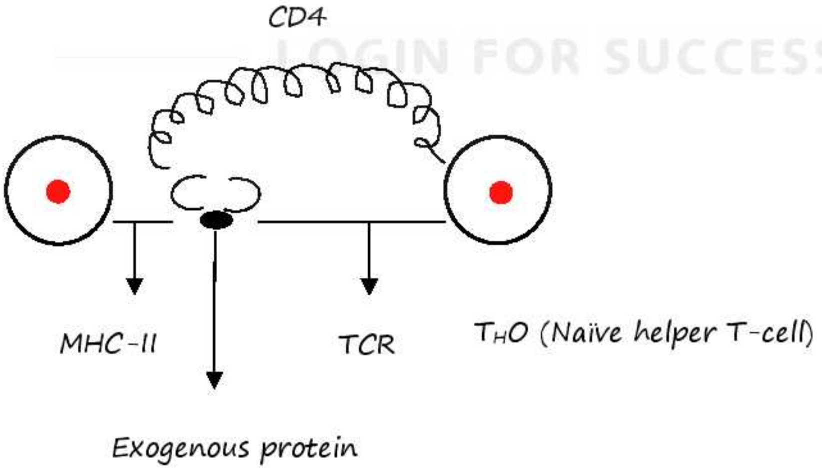

CD4
MHC-II
TCR
$T_H0$ (Naïve helper T-cell)
Exogenous protein

**Exogenous protein:**

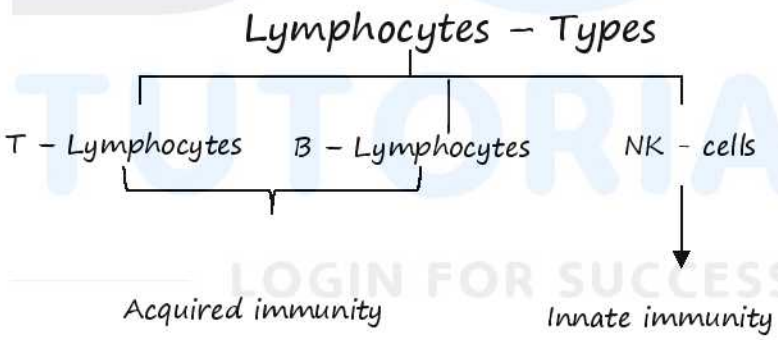
- Comes from degradation/Breakdown of a microbe

**Endogenous protein:**

- Comes when the microbe multiplies in a host cell and forms its own proteins inside host cell. These proteins are endogenous proteins
- MHC-II are proteins expressed on the surface of APCs
- CD$_4$: are markers of helper T-cells/T$_H$-Cells
- CD$_8$: are markers of cytotoxic T cells/T$_c$ cells

## Natural Killer cells (NK cells) 00:37:37

- Are licensed to kill
- Also known as Large Granular Lymphocytes (LGL)
- Granules have → Perforins & Granzymes


Lymphocytes – Types
T – Lymphocytes
B – Lymphocytes
NK - cells
Acquired immunity
Innate immunity

- Also known as NULL cells
- CD markers of NK cells: CD16; CD56. CD16: It is a receptor for Fc part of IgG
- NK cells kill all kinds of microbes, but have excellent activity against – Tumor cells & Virus-infected cells
- They are not tumor specific or virus specific

## Mechanism of killing the target cell:

- Engagement of fas –Protein on the target cell by FAS – Ligand on NK cell (Fas-L)

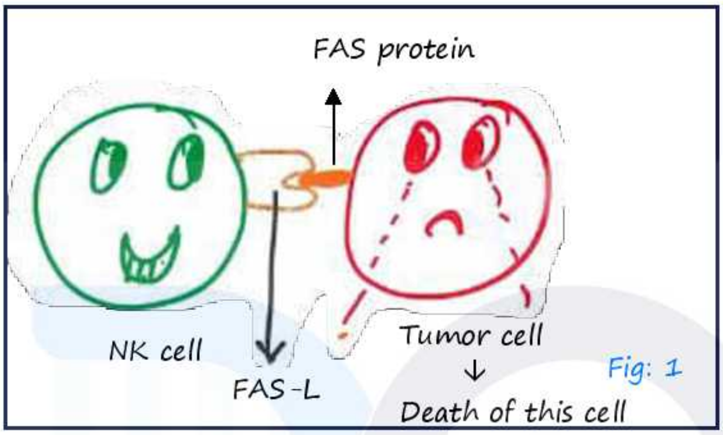

FAS protein
NK cell
FAS-L
Tumor cell
↓
Death of this cell

Fig: 1

- ADCC (Antibody dependent cell mediated cytotoxicity):

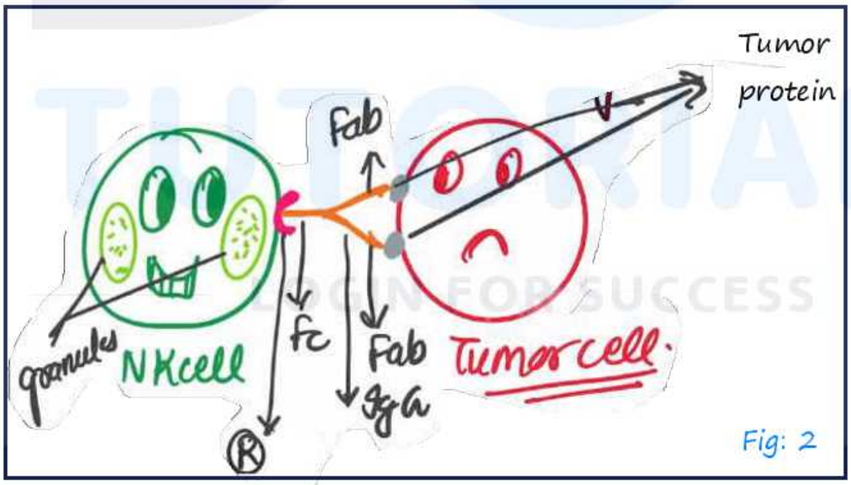

Tumor protein
Fab
granules
NKcell
fc
Fab
IgG
Tumor cell
Ⓡ

Fig: 2

## Granular contents are:

- Perforins – Perforate/Insert holes in the target cell, Kill from outside

- Granzymes -Enter the target cell through holes made by perforins & activate apoptosis, Kill from inside

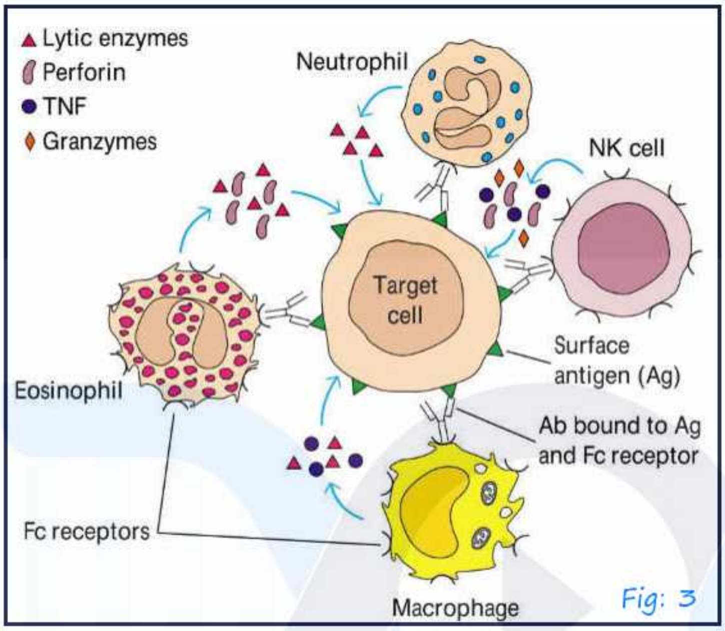

▲ Lytic enzymes
Perforin
● TNF
♦ Granzymes
Neutrophil
NK cell
Target cell
Surface antigen (Ag)
Eosinophil
Ab bound to Ag and Fc receptor
Fc receptors
Macrophage

Fig: 3

Cells which participate in ADCC:

- Macrophages
- Eosinophils
- Neutrophils
- NK cells

There is no phagocytosis during ADCC

TNF: present in granules of Macrophages + Neutrophils

NKcells kill those cells which show down regulation/Decreased expression of MHC - I

- Normally MHC-I protein is expressed on the surface of all nucleated cells (Except sperm cells) & Platelets

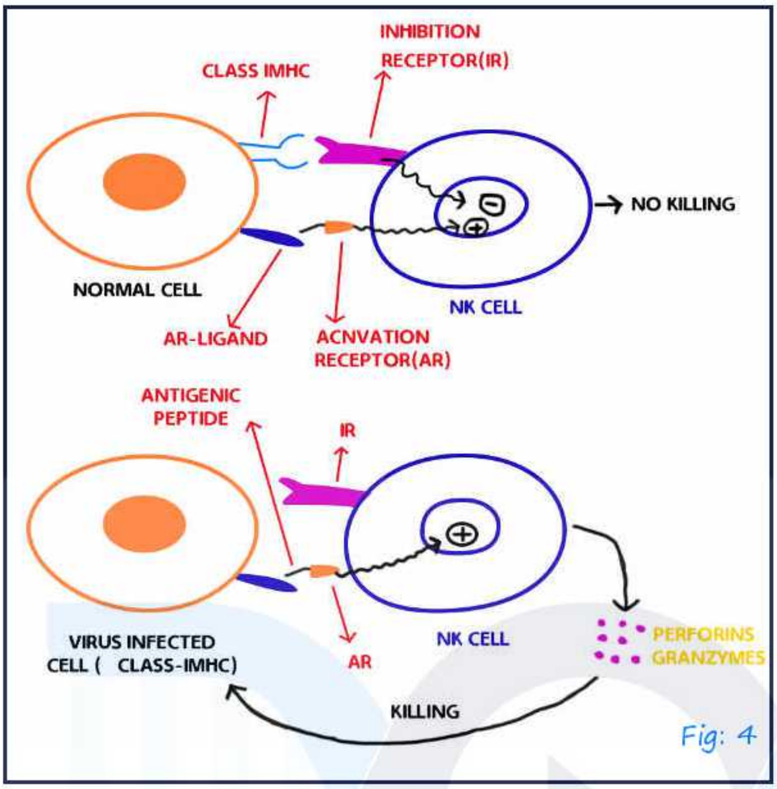

INHIBITION RECEPTOR(IR)
CLASS IMHC
NO KILLING
NORMAL CELL
NK CELL
AR-LIGAND
ACNVATION RECEPTOR(AR)
ANTIGENIC PEPTIDE
IR
VIRUS INFECTED CELL ( CLASS-IMHC)
AR
NK CELL
PERFORINS
GRANZYMES
KILLING

Fig: 4

## Humoral members of innate immunity 01:01:08

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- Lysozyme
- CRP
- Interferon
- Complement

## Lysozyme 01:08:58

- Discovered by Fleming
- Present in body secretions Except:
  - CSF
  - Urine
  - Sweat
- It has antibacterial action by breaking down the peptidoglycan cell wall

## Interferons 01:10:33

- They are a type of cytokines
- Chemically interferons are glycoproteins
- Interferon – Classification:

| **Type – 1 IFNs** | **Type – 2 IFNs** |
|---|---|
| eg: IFN – Alpha<br>IFN – Beta | eg: IFN – $\gamma$ |
| - They have antiviral action | - Also known as immune IFN<br>- Produced by $T_{H1}$ cells & NK cells<br>- It takes part in immune response<br>- IFN-$\gamma$ have no antiviral action |

IFN alpha:

- Produced by leukocytes

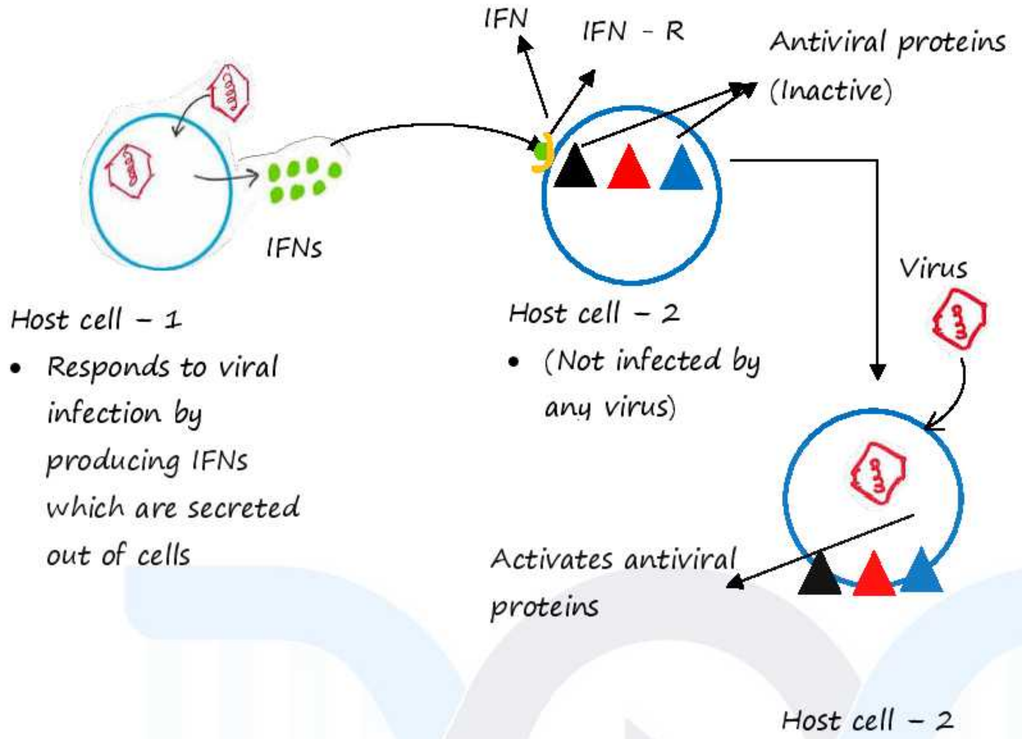
IFN Beta:

- Produced by fibroblasts

> Most important source of antiviral IFNs:
> plasmacytoid dendritic cell (PDC)

- Most of our body cells have receptors for IFNs

## Mechanism of antiviral action of type -I Interferons:


IFN
IFN - R
Antiviral proteins
(Inactive)
IFNs
Host cell – 1
• Responds to viral infection by producing IFNs which are secreted out of cells
Host cell – 2
• (Not infected by any virus)
Virus
Activates antiviral proteins
Host cell – 2

- IFNs produced by a cell are not going to act on the same cell but they bind to IFN-® present on neighbouring cells

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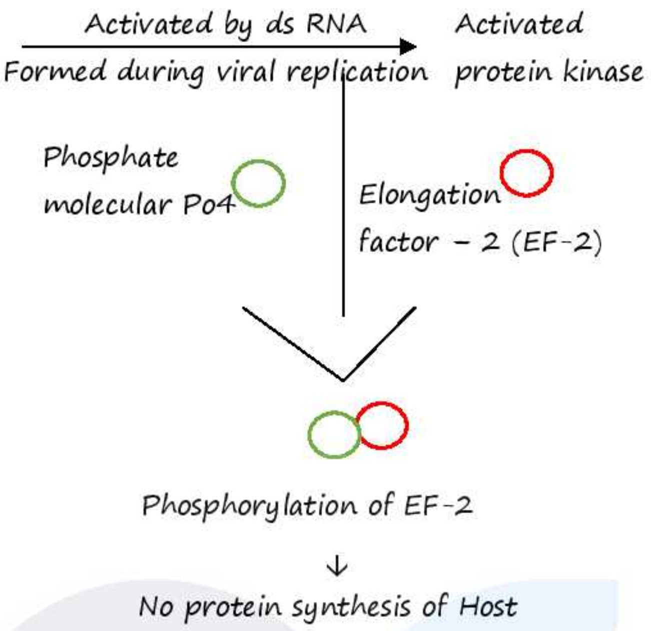
## Anti-viral proteins:

- Δ – Inactive -oligo A oligo synthetase $\xrightarrow[\text{During viral replication}]{\text{DsRNA produced}}$ Active – A Synthetase
  ↓
  Oligo – A molecule is formed

- Δ – Inactive Ribonuclease Ribonuclease $\xrightarrow{\text{Oligo – A}}$ Active
  ↓
  Breaks viral & cellular mRNA

- Δ – Inactive protein Kinase


Activated by ds RNA
Formed during viral replication
Activated protein kinase
Phosphate molecular Po4
Elongation factor – 2 (EF-2)
Phosphorylation of EF-2
No protein synthesis of Host

**End result:**

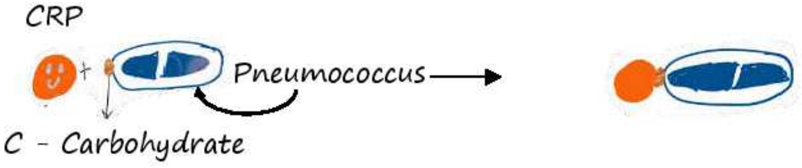
- Death of the virus-infected cell
- Antiviral activity of Type II IFN (IFN – Alpha, Beta) is due to:

  Inhibition of viral translation – i.e. inhibit viral protein synthesis

## C-Reactive Protein (CRP) 01:34:24

- Beta – globulin protein made by liver cells
- It reacts/binds with "C" carbohydrate antigen of Pneumococcus in vitro

C in CRP for carbohydrate


CRP
+
Pneumococcus
C - Carbohydrate

- CRP levels are increased in acute inflammation (Acute phase reactant)
- Used to monitor activity of autoimmune diseases e.g. SLE, RA
- CRP is not an antibody to Pneumococcus (Because CRP is a Beta globulin whereas Abs are γ-globulins)
- CRP is measured by:

  Agglutination test (More sensitive)

  Precipitation test

# MEMBERS OF ACQUIRED IMMUNITY

## Members of Acquired immunity 00:00:08

Members of Acquired immunity

Cellular

↓

T-cells/T-lymphocytes

Helper/inducer T-cells ($T_H$ - Cells)

Cytotoxic T-cells (Tc-cells)

Humoral

↓

- Antibodies (Y)
- Plasma cells
- B-cells

- CD3 is the pan – T-cell marker
- CD2 is less important pan – T-cell marker
- Both $T_H$ cells and Tc – cells are positive for CD3 MARKER

T-cells classification

$T_H$

↓

CD3 positive

CD4 positive

$T_c$

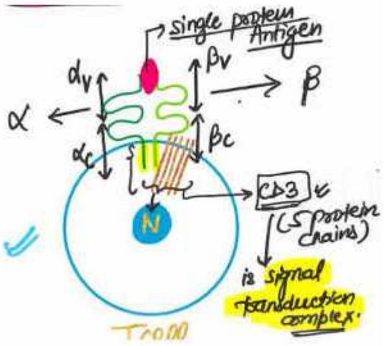
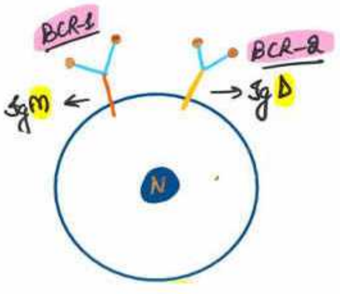
↓

CD3 positive

CD8 positive

- Humoral: Antibodies
- Plasma cells are differentiated B-cells
- One plasma cell produces around 2000 antibody molecules/second
- Plasma cells have very well developed golgi apparatus

## T-cell and B-cell Receptors 00:09:54

| **T-cell receptor (TCR)** | **B-cell receptor (BCR)** |
|---|---|
|  single protein Antigen<br>$\alpha_v$<br>$\beta_v$<br>$\beta$<br>$\alpha$<br>$\alpha_c$<br>$\beta_c$<br>CD3<br>(5 protein chains)<br>is signal transduction complex.<br>N<br>T cell |  BCR-1<br>BCR-2<br>SgM<br>Sg D<br>N<br>B cell |
| TCR Complex = TCR+ CD3 | BCR is antibody molecule attached to cell membrane of B-cell/sIg /mIg<br>sIg = surface Ig<br>mIg = membrane Ig |
| 1TCR can bind to a single antigen which should be protein only | 1BCR can bind to two identical antigens which could be:<br>-proteins<br>-carbohydrates<br>-lipids<br>-nucleic acids |

- CD markers on B-cells: CD19
  CD20
  CD21

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| T-cells | B-cells |
|---|---|
| They mature in thymus | They mature in bursa of Fabricius present in birds |
|  | In humans, site of maturation is bone marrow |

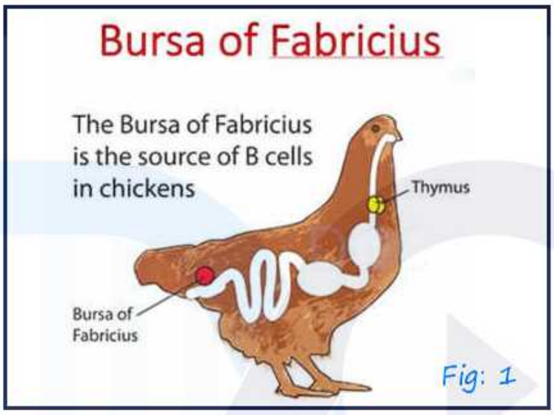

Bursa of Fabricius
The Bursa of Fabricius is the source of B cells in chickens
Thymus
Bursa of Fabricius

Fig: 1

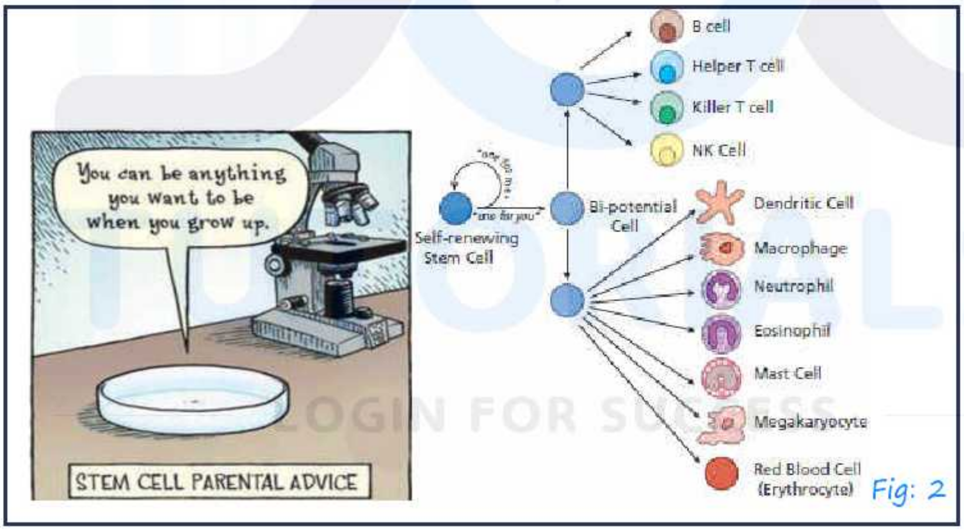

You can be anything you want to be when you grow up.
STEM CELL PARENTAL ADVICE
"one for me"
"one for you"
Self-renewing Stem Cell
Bi-potential Cell
B cell
Helper T cell
Killer T cell
NK Cell
Dendritic Cell
Macrophage
Neutrophil
Eosinophil
Mast Cell
Megakaryocyte
Red Blood Cell (Erythrocyte)

Fig: 2

## Lymphoid organs 00:32:48

- They are the aggregations / collections of lymphocytes
- Lymphoid organs:

| **Primary/central** | **Peripheral/secondary** |
|---|---|
| They are organs where immune cells mature | They are organs where mature immune cells (T and B-cells) stay and wait for their antigen |
| Example: Bone marrow,<br>-thymus. | Example: Spleen<br>- Lymph nodes<br>-Mucosa Associated Lymphoid Tissue (MALT) |

**Spleen:** The largest secondary lymphoid organ; also overall, the largest lymphoid organ

Spleen
- T-cells → periarteriolar sheath
- B-cells → germinal Centre

Lymph node
- T-cells → paracortex
- B-cells → cortex

## Activators of T-cells and B-cells 00:36:44

Activators of T-cells and B-cells

| **T-cells** | **B-cells** |
|---|---|
| ↓ | ↓ |
| Concanavalin | EBV (Epstein Barr virus) |
| Phytohaemagglutinin | LPS |

# ACTIVE AND PASSIVE IMMUNITY

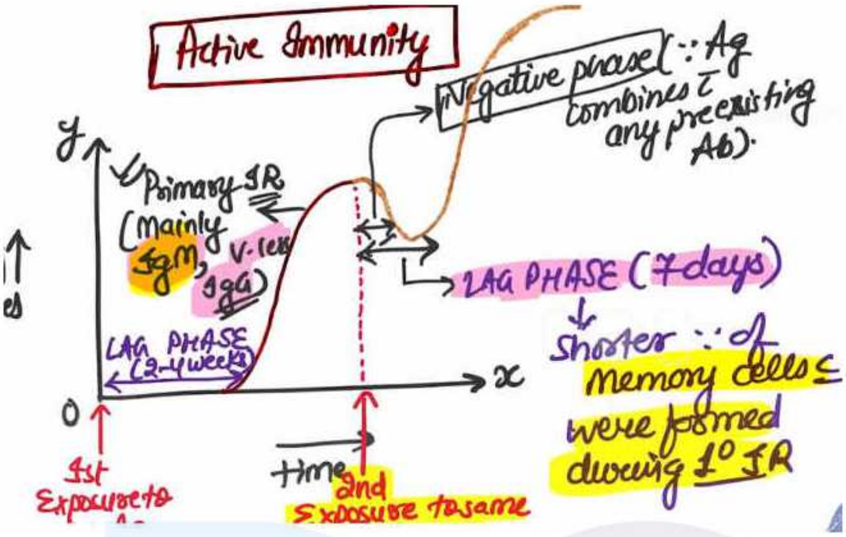
## Classification of Acquired/Adaptive immunity

00:00:42

- Acquired/adaptive immunity are divided into two types:
  1. Active immunity
  2. Passive immunity

| Active immunity | Passive immunity |
|---|---|
| Immune system does active work<br>Example: in case of an infection | Administration of antibodies from some source |
| Types:<br>Natural ↓ Natural infection<br>Artificial ↓ vaccines | Types<br>Natural ↓<br>i. Transplacental transfer of IgG<br>ii. IgA antibody present in:<br>• Breast milk<br>• colostrum<br>Artificial ↓<br>Commercially available antisera<br>Example: -HBIG<br>antisnake venom |
| It takes time | It provides immediate protection |
| Memory cells are formed | No memory (lasts as long as antibody persists) |
|  | Passive immunity can be relied upon in case of immunocompromised people |

## Active immunity 00:08:50



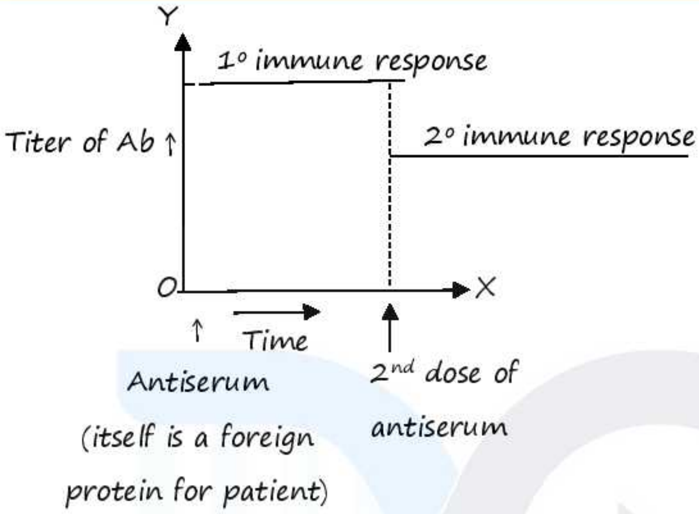
### Active immunity

| Primary | Secondary |
|---|---|
| Longer lag phase | Shorter lag phase |
| Mainly IgM response | Mainly IgG response |
| Less titer of antibodies | Higher titer of antibodies |
| No negative phase | Negative phase may be present |
| Lesser affinity/avidity of antibody for Ag | More affinity/avidity of antibody for antigen |

- Affinity: strength of interaction of antibody with antigen at a single point
- Avidity: strength of interaction of antibody with antigen at multiple points

- IgG: better affinity than IgM
- IgM: Better avidity than IgG

## Passive immunity 00:21:40


Y
1° immune response
Titer of Ab ↑
2° immune response
O
X
↑
Time
Antiserum
(itself is a foreign
protein for patient)
2nd dose of
antiserum

Some Antibodies clear due to immune elimination/immune clearance

1) Anti-serum – Dose 1 → Is foreign therefore immune system produces

Antibody against them

YYYYY

(Foreign to patient)

2) Anti-serum Dose 2 → YYY remain because of immune elimination/immune clearance

YYYYY

YY (antibodies against antiserum)

# ANTIGEN

## Antigen 00:00:26

- Antigen is a foreign substance/molecule which when enters the body, stimulates the immune system to mount an immune response

### 2-properties of a good antigen

- Immunogenicity - property to induce an immune response
- Antigenicity – property of an antigen to bind with the antibody/ immunocompetent cells

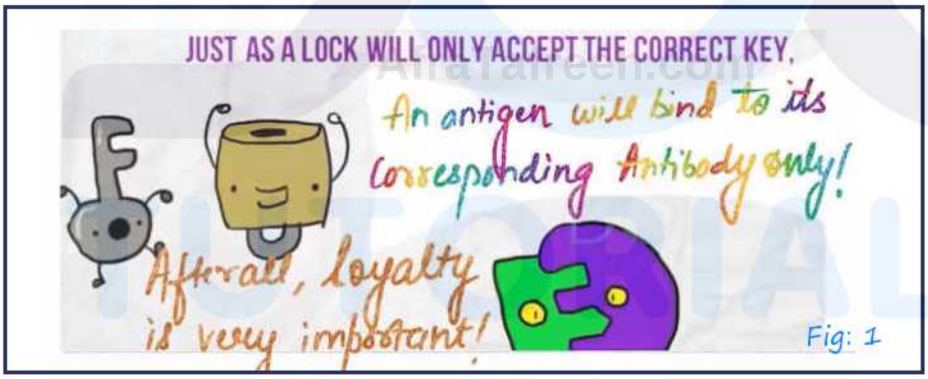

JUST AS A LOCK WILL ONLY ACCEPT THE CORRECT KEY,
An antigen will bind to its corresponding Antibody only!
Afterall, loyalty is very important!

Fig: 1

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- Ag-Ab interaction is very specific
- This is known as the specificity of Ag-Ab reaction

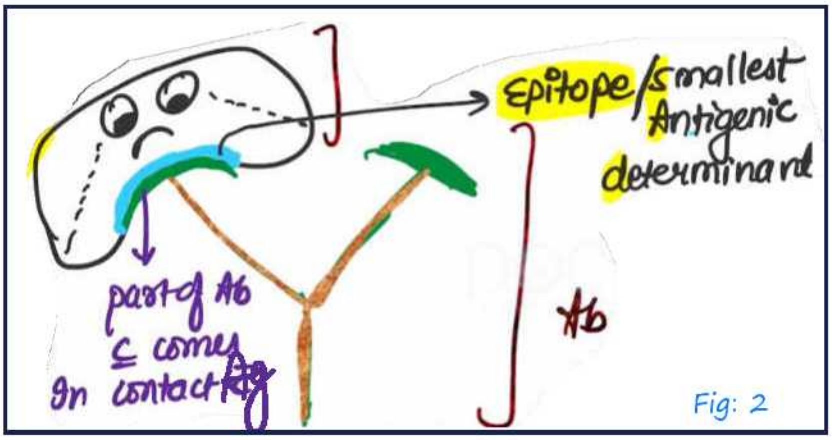
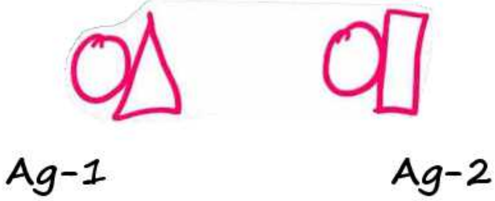

Epitope/smallest Antigenic determinant
part of Ab c̲ comes in contact Ag
Ab

Fig: 2

- Sometimes specificity of Ag-Ab reaction fails which may lead to disease



- Ag-1 should bind to Ab-1
- Ag-2 should bind to Ab-2

Due to failure of specificity of Ag-Ab reaction disease are caused eg: Acute rheumatic fever

- This is due to molecular mimicry

---

## Hapten/proantigen/incomplete antigen

00:15:35

---

- It is generally a low molecular weight substance which lacks the property of immunogenicity, but has the property of antigenicity
- It can be converted into a complete Ag by tagging/attaching it to a protein carrier

**Body response towards hapten:**

| | | | |
|---|---|---|---|
| I | Hapten | Injected into → a person | No immune response |
| II | Hapten Tagged to | Injected into → same person | 1° immune response |

| | | | |
|---|---|---|---|
| | Protein carrier | | IgM against 'H'<br>IgM against 'P' |
| III | Hapten | Is 2° immune<br>→<br>response possible? | No<br>But 'H' binds to any pre-existing IgM if it present (Due to property of antigenicity) |
| IV | H<br>Plus<br>P | Is 2° Immune<br>→<br>response possible? | Yes<br>IgG forms against 'H'<br>IgG forms against 'P' |

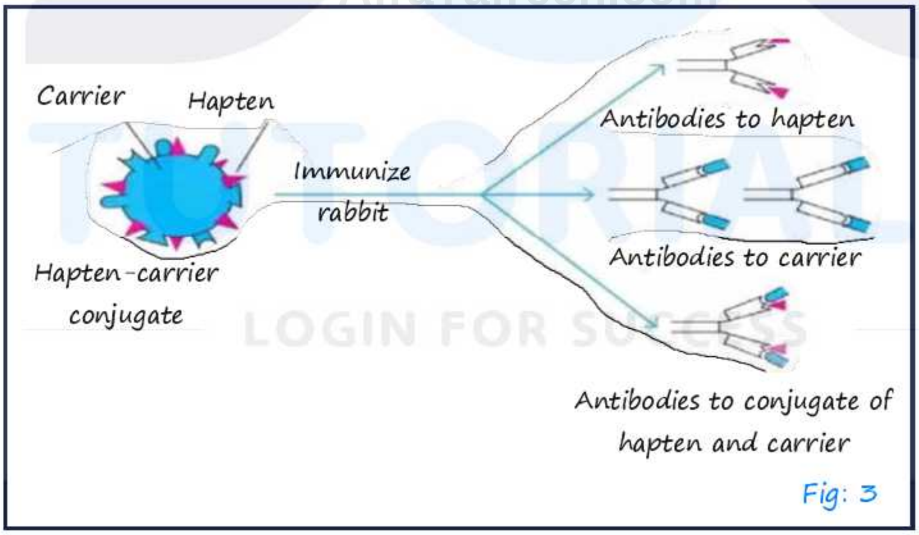

Carrier
Hapten
Hapten-carrier conjugate
Immunize rabbit
Antibodies to hapten
Antibodies to carrier
Antibodies to conjugate of hapten and carrier

Fig: 3

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## Determinants of a strong/good antigen

00:21:20

i. Chemical nature:

Proteins > Carbohydrates > Lipids > Nucleic Acids

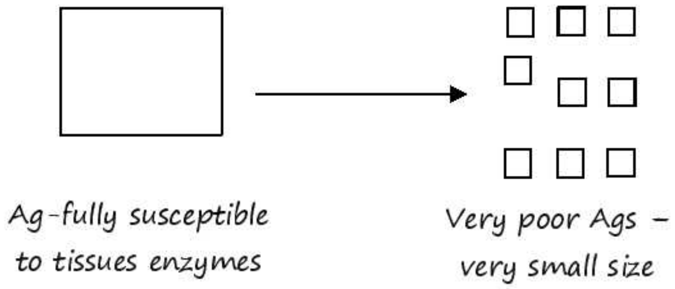
ii. Very big or very small molecules are poor antigens

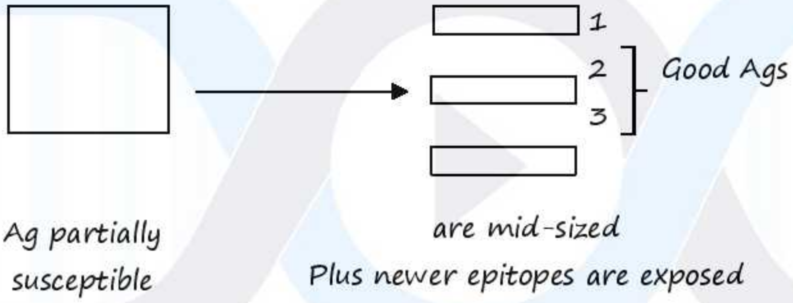
- Midsized molecules good antigens

iii. Degree of foreignness

- The more foreign a molecule, the better antigen it is

iv. Susceptibility to tissue enzymes


Ag-fully susceptible to tissues enzymes
Very poor Ags – very small size


1
2
3
Good Ags
Ag partially susceptible
are mid-sized
Plus newer epitopes are exposed

## Specificity of an Ag

00:29:48

i. Species specificity

- Ags specifically present in all members of a species
- Xenograft: Almost always rejected

ii. Organ specificity

- Ags specificity present in a specific organ in different species
- Eg: Brain tissue Ag of:
  - Humans
  - Mice

iii. Heterophile specificity

- Similar Ags present in different species & need not be organ specific
- Example:
  a. For M.pneumoniae infection tentative diagnosis we do:
    - Cold agglutinin test
    - Streptococcus MG test

  b. Paul Bunnell Test for EBV

  c. Weil felix reaction for rickettsiae

iv. Individual specificity
- All of us are antigenically unique
- Antigens specifically/uniquely present in each member of a species
- Best example: MHC/HLA proteins

↓

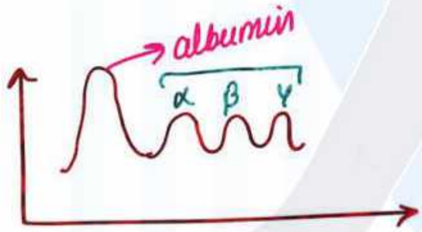
**Gene is present on 6p chromosome**

v. Iso-specificity
- Variation of a group of antigens present in different members of a species
- Example:
  - ABO blood group of antigens
  - Rh Ag

# ANTIBODY PART - I

## Antibody (Ab) 00:00:41

- It is a substance which combines/reacts with Antigen (Ag) to eliminate an Infectious agent/to activate an Immune cell to generate some response
- Antibodies – They are Immunoglobulins
- When serum proteins are fractionated according to mass & charge:



αβγ = Globulins

Alb: Globulin = 1.7:1

Antibody: γ- globulin

- All Abs are γ- globulins but
  All γ – globulins are not Abs
- Structurally they are Glycoproteins
  - Protein part is responsible for most of the functions of an Ab molecule.
  - Abs are primarily proteins, with less amount of sugar

## Structure of Monomeric Ab/Ab-monomer 00:08:22

- Single molecule of Ab

- H-Heavy chains

  Mol wt. is ≈ 50,000 Daltons
- 2 'H' chains are identical
- 2 'L' chains are identical
- Molecular weight of 1 light chain – 25,000 daltons
- They are connected by disulphide bonds (-S-S-)
- 2 Terminals are present: $NH_2$ – amino terminal and

  COOH – carboxyl terminal.
- Genes for 'H' chains present on Chromosome – 14
- 'L' chains are of 2 types

  Kappa (K)  Lambda($\lambda$)
- Ratio of K: $\lambda$ in human serum 1.5:1

- 1 Antibody molecule has either

  both $\lambda$ light chains or

  both K light chains

  But never 1 K and 1 $\lambda$

- Genes of K light chain present of chromosome – 2
- Genes for $\lambda$ light chain present on chromosome – 22
- Each light chain has 2 parts

  - $V_L$ – variable part of 'L' chain
  - $C_L$ – Constant part of 'L' chain

- Similarly, each heavy chain has many parts

  $V_H$ – Variable part of 'H' chain

  $CH_1$l, $C_H2$, $C_H3$ – constant parts of heavy chain

## Classes of Antibodies 00:18:53

IgG

IgA

IgM

IgD

IgE

IgM & IgE – Have longer 'H' chains
∴ Have and additional '$C_H$' domain.
[$C_H1$, $C_H2$, $C_H3$, $C_H4$]

- IgM & IgE – do not have Hinge region
- IgG, IgA, IgD – have 3 $C_H$ regions

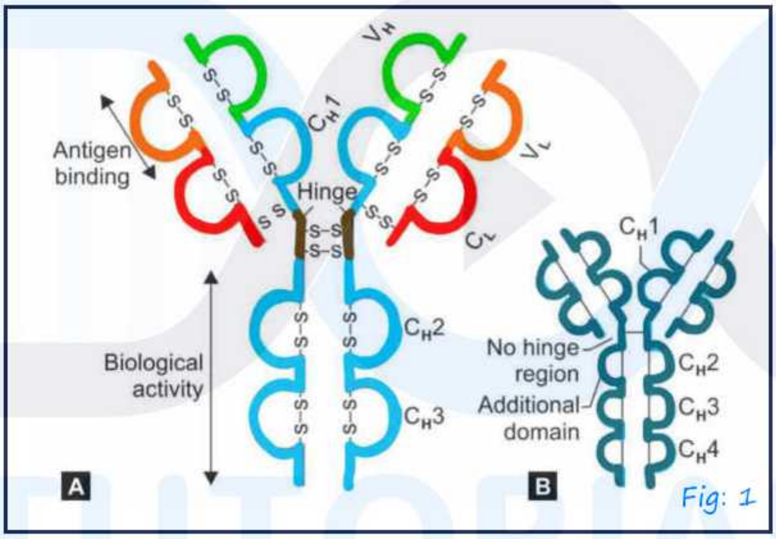

V_H
C_H1
V_L
C_L
Antigen binding
Hinge
S-S
C_H2
C_H3
Biological activity
A
B
C_H1
No hinge region
Additional domain
C_H2
C_H3
C_H4

Fig: 1

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- Hinge region is rich in 2 amino acids – Proline & cysteine
  - It provides flexibility to Ab-molecule, so that Ab can easily bend & approach the Ag.
  - Both $V_H$ & $V_L$ on one side form the Ag binding site.
  - $V_H$ alone or $V_L$ alone can't bind to Ag
  - 1 $V_H$ $V_L$ region binds to 1 Ag molecule.
  - Valency of an Ab molecule = No. of $V_H$ $V_L$ regions in the Ab

or

Valency of an Ab = No. of Ags an Ab molecule can bind

∴ Valency of Monomeric Ab = 2

- Within the variable regions, Hypervariable regions/Hotspots/Complementary Determining Regions (CDRs) where the exact/precise bond forms between Ag and Ab

  Bonds between Ag & Ab:

  Hydrogen bonds

  - $H_2$

  Hydrophobic interactions

  - I – Ionic bonds/Electrostatic force
  - V – Vander wall forces

- No covalent bond is formed ∵ they are irreversible
- All the above 4-bonds are reversible but form firm interaction between antigen and antibody

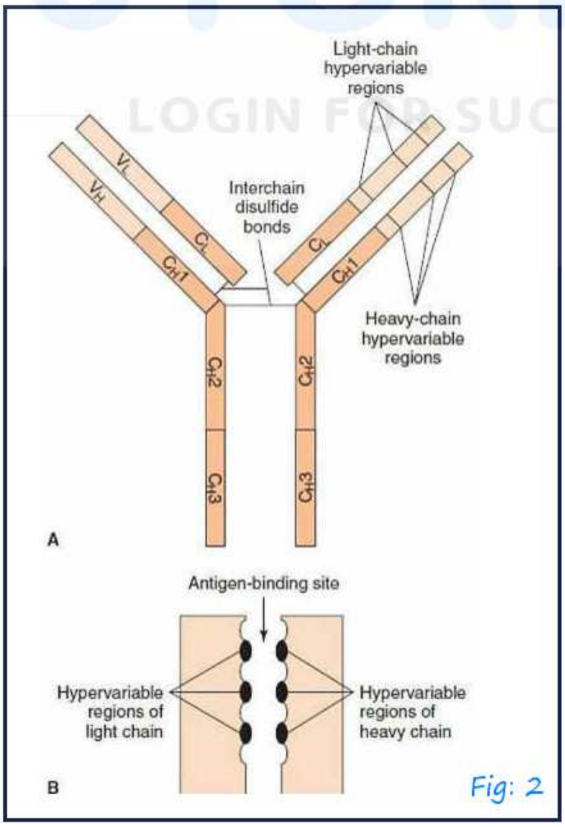

Light-chain hypervariable regions
$V_L$
$V_H$
Interchain disulfide bonds
$C_L$
$C_H1$
$C_L$
$C_H1$
Heavy-chain hypervariable regions
$C_H2$
$C_H2$
$C_H3$
$C_H3$
A
Antigen-binding site
Hypervariable regions of light chain
Hypervariable regions of heavy chain
B

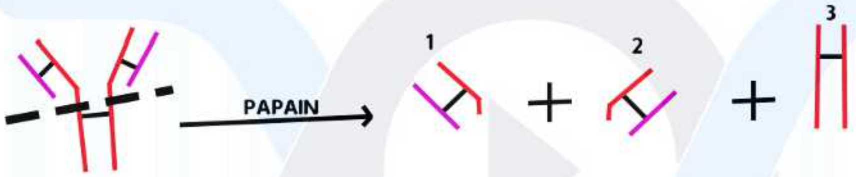
Fig: 2

- $F_{ab}$ – Fragment Antigen Binding
- $F_c$ – Fragment crystallisable

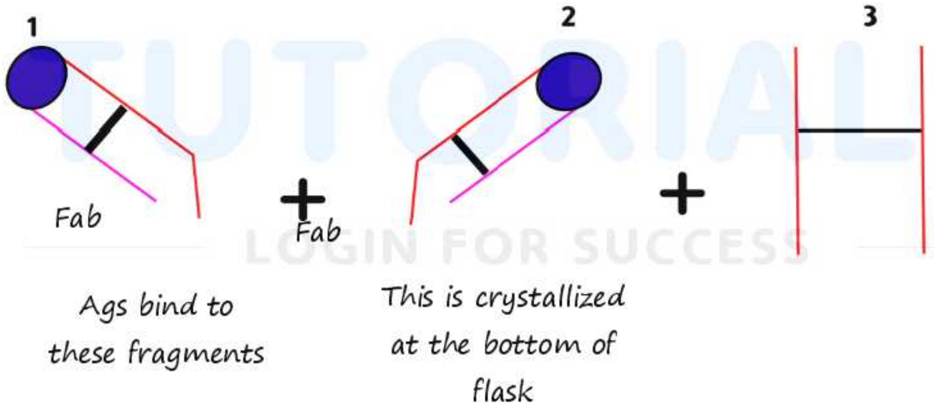
## Effects of Proteolytic Enzymes (Papain & Pepsin) on Ab-molecule: 00:39:40

### I. Effect of papain enzyme on Ab:

- Papain attacks the antibody molecule above the Inter – H – chain disulphide bond



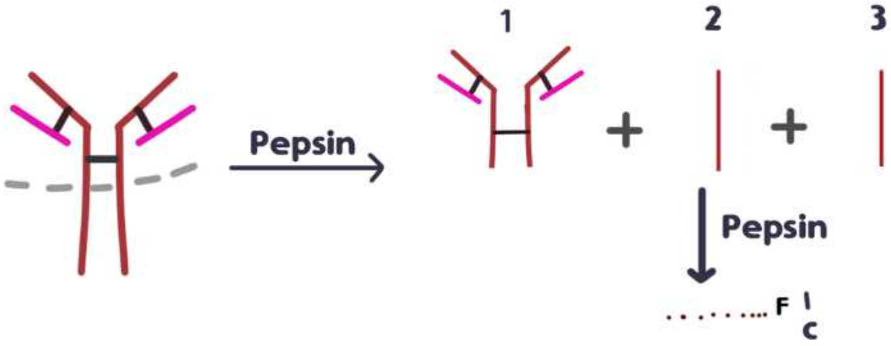
- Now Ag is added:


1
2
3
Fab
Fab
+
+
Ags bind to these fragments
This is crystallized at the bottom of flask

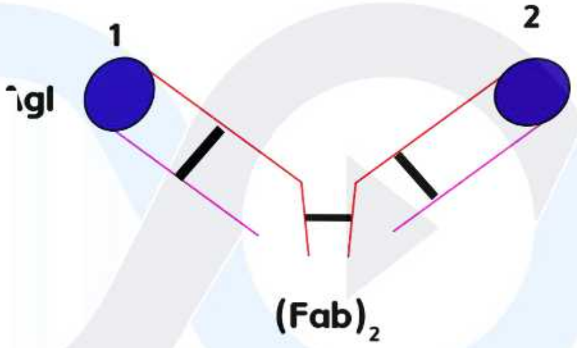
- ∴ Due to action of papain = 2 Fab fragments + 1 $F_c$

### II. Effect or action of pepsin on Ab-molecule:

- Pepsin attacks the Ab below the Inter – H – chain disulphide bonds.


Pepsin
1
2
3
+
+
Pepsin
F
c

- When Ag is added:



– Action of pepsin on Ab molecule

$(fab)_2 + f_c$

# ANTIBODY PART - II

## Class/Isotype/Isospecificity of Ab 00:00:15

- This is based on differences in $C_H$ region
- $C_H$ region is of 5 types
- Therefore 5 classes of antibodies are present

| Ig class | IgG | IgA | IgM | IgD | IgE |
|---|---|---|---|---|---|
| CH region | $\gamma$ | $\alpha$ | $\mu$ | $\delta$ | $\varepsilon$ |
| IgG> IgA > IgM > IgD > IgE: order of serum concentration | | | | | |

i. IgG: 4 – subclasses (Based on $C_H$ region differences)

IgG1 > IgG2 > IgG3 > IgG4 → serum concentration

ii. IgA: 2 – subclasses (Based on $C_H$ region differences)

Serum concentration: IgA1 > IgA2

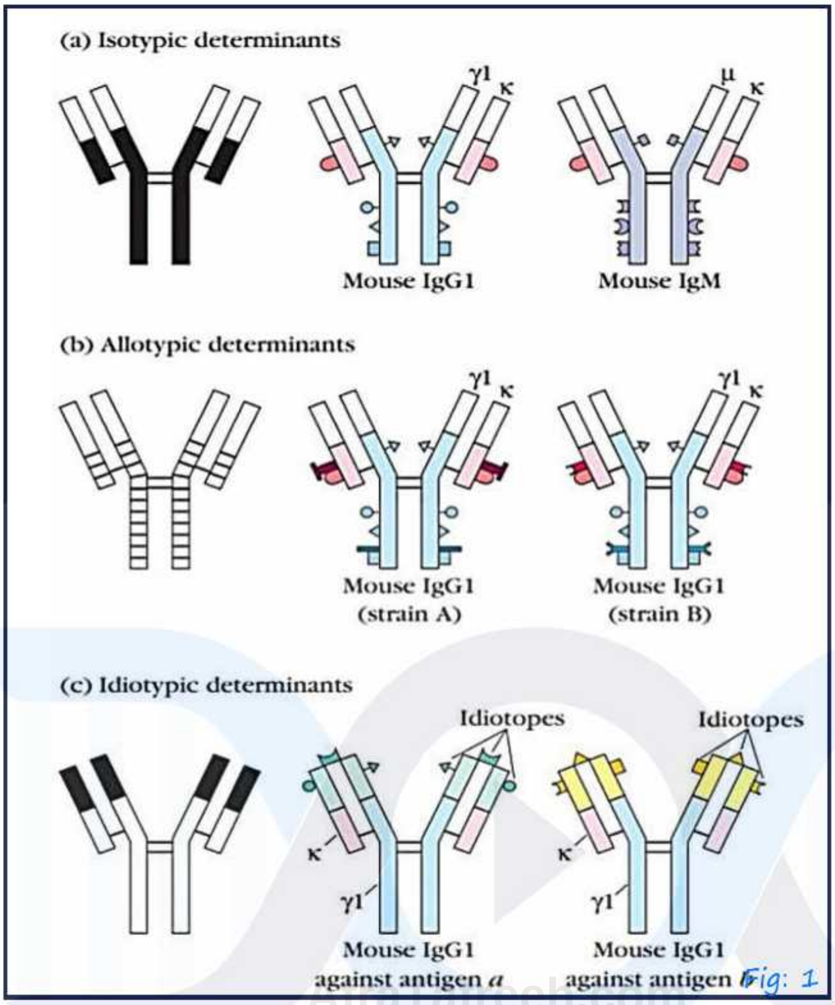

(a) Isotypic determinants
γ1
κ
μ
κ
Mouse IgG1
Mouse IgM
(b) Allotypic determinants
γ1
κ
γ1
κ
Mouse IgG1
(strain A)
Mouse IgG1
(strain B)
(c) Idiotypic determinants
Idiotopes
Idiotopes
κ
γ1
κ
γ1
Mouse IgG1
against antigen a
Mouse IgG1
against antigen b

Fig: 1

## Allotype determinants 00:11:50

- The subtle differences in the amino acid sequences in the $C_H$ region of a particular class of an Ab in different members of same species
- Reason: multiple alleles
- Allotype: sum total of all the allotypic determinants
- They are seen in
  - IgG – 25 types
  - IgA
  - Kappa 'L' chain – 3 types

## Significance:

- Allotypic determinants were earlier analyzed to solve cases of paternity disputes

## Light chains:

- Light chains (K, λ) – based on C region difference

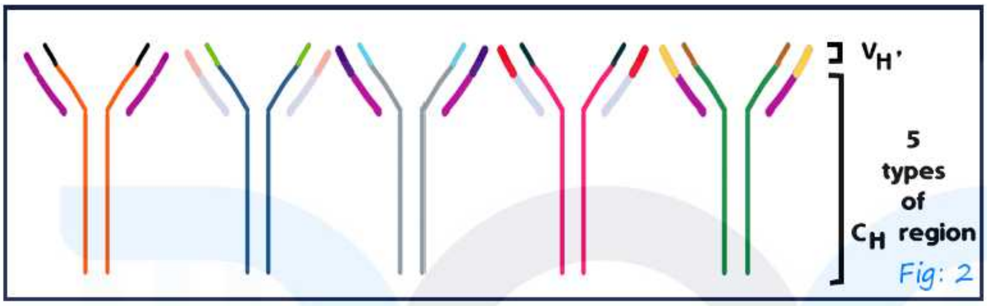

$V_{H}$'
5 types of $C_H$ region

Fig: 2

# ANTIBODY PART - III

## Allotype 00:06:11

- Refers to subtle differences in the amino acid sequences present in different molecules of same class of an antibody present in different members of species.

## Idiotypic Determinants/Idiotopes 00:10:50

- Refers to the variations present in variable region of Ab molecule

## Classes of Antibodies 00:13:10

IgM:

- It is an Ab of acute/recent infection.
- It is a millionaire molecule – $10^6$ daltons
- It is a pentameric molecule.
- 'J'/Joining chain is present, connecting different monomers, J-chain is present in polymeric antibodies
- Valency of IgM:10

  But effective valency is 5

  This is because of steric hindrance
- 80% of IgM is Intravascular
- Only Ab class made by fetus, i.e, made before birth (≈ 20 weeks of gestation)

- IgM is present on B-cells as B-cell receptor (BCR) as a monomer
- IgM: known as agglutinating Ab, it has 20 times more agglutinating ability than IgG
- IgM is the most potent activator of classical pathway of complement activation
- It is known as Lytic Ab
- Due to complement activation, membrane attack complex (MAC) forms which causes lysis of the cell

IgG:

- Cross the placenta (except $IgG_2$)
- Most abundant Ab class in the serum
- Half life = 23 days
- IgG signifies past/chronic infection
- There are 4-subclasses; order of concentration: $IgG_1 > IgG_2 > IgG_3 > IgG_4$
- IgG is the 2nd most potent activator of classical pathway of complement
- $IgG_4$ does not activate classical pathway but activates the alternate pathway of complement
- IgG: precipitating Ab
- It is a monomer, valency is 2
- IgG – very good opsonin
- $IgG_1$ & $IgG_3$ are excellent opsonins (better than IgG2 and IgG4)

## IgA:

- It activates alternate pathway of complement
- It has 2 subclasses: $IgA_1 > IgA_2$
- It can exists in 2 forms''
  a. Monomer – present mainly in serum
  b. Dimer/Dimeric/Secretory Ab → present in secretions, mucosal surfaces
- Dimeric form of IgA is known as Local/Mucosal Ab/Mucosal Antiseptic paint

- Structure of Secretory IgA:
  - J-chain is present connecting the monomers
  - Both the monomers and J-chain are produced by plasma cell
  - Secretory component/Secretory piece is present which is produced by mucosal epithelial cell derived from poly-Ig receptor

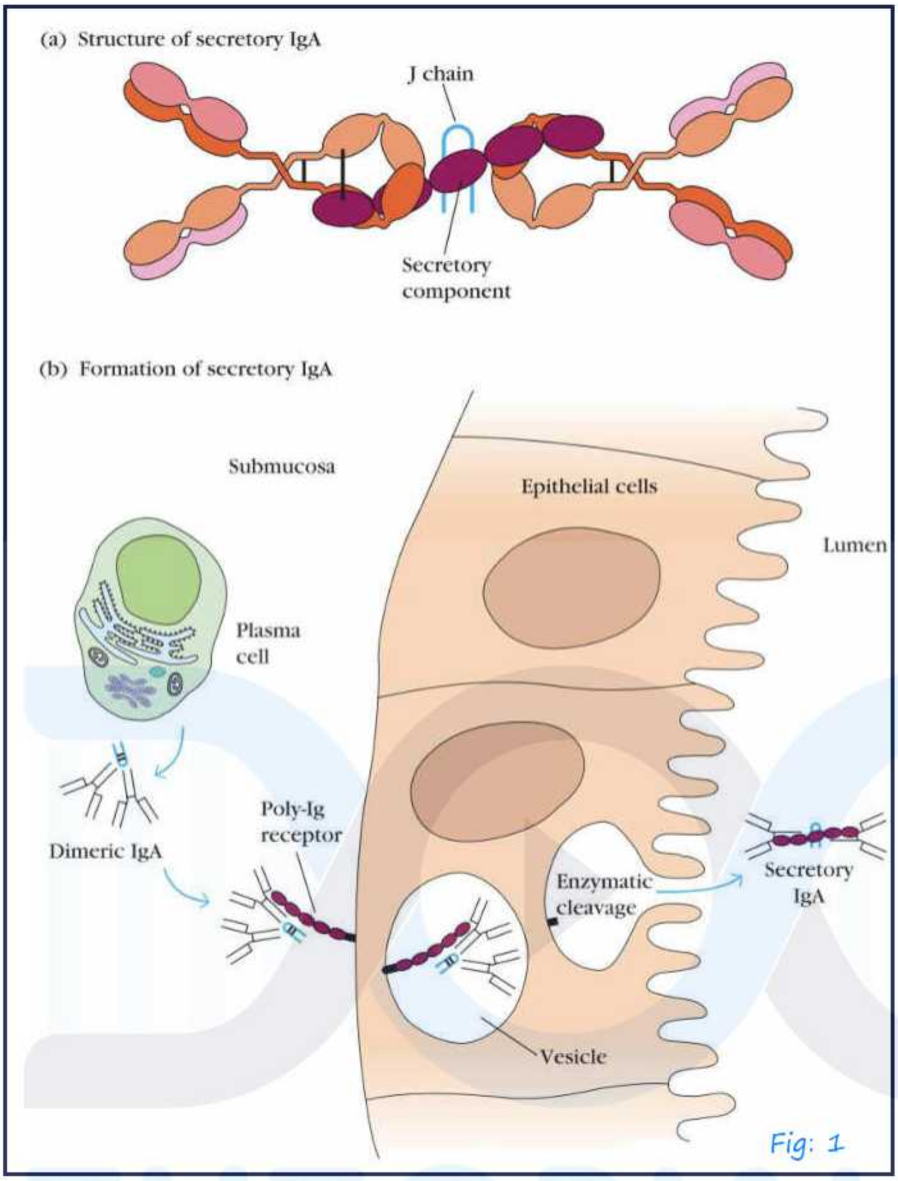

(a) Structure of secretory IgA
J chain
Secretory component
(b) Formation of secretory IgA
Submucosa
Epithelial cells
Lumen
Plasma cell
Dimeric IgA
Poly-Ig receptor
Enzymatic cleavage
Secretory IgA
Vesicle

Fig: 1

- Functions of secretory piece:
  - i. Protects the secretory IgA molecule against attack by bacterial proteases in the gut
  - ii. Helps in transport of IgA to the lumen
    - IgA is the most abundant Ab-class in breast milk & colostrum

## IgD:

- Present on B-cell surface as BCR
- Free IgD does not bind to Ag

- IgD has got the 2nd highest carbohydrate content amongst all classes
- It activates the alternate pathway of complement

**IgE:**

- It has the least concentration in serum
- It has shortest half life
- Most heat-labile of all classes
- It is also known as Homocytotropic antibody. i.e., it binds to mast cell & basophils in humans because these cells have receptors for $F_c$ part of IgE
- IgE plays an important role in Type – I Hypersensitivity reaction
- It is also known as reaginic antibody
- It has got the highest carbohydrate content

# COMPLEMENT PROTEINS PART – I

## Complement Proteins: 00:00:17

- Definition: It is a group of proteins present in serum which help in acquired immune response & inflammatory response.
- Most are in the form of inactive enzymes or pro-enzymes.
- There are nearly 20 complement proteins.
- Most of these are produced by hepatocytes, some are also produced by
  - Blood monocytes
  - Tissue macrophages
  - Epithelial cells of GIT & Genito urinary tract
- Complement activation/Complement fixation/Complement utilization/Complement consumption :all means the same.
  - This can occur by 3 pathways.
    i. Classical pathway
    ii. Alternate pathway
    iii. Lectin pathway

### I. Classical Pathway

- It was discovered first.
- It is activated by not Ag alone or Ab-alone but it is activated by Ag-Ab complexes

- The Ag-Ab complexes have
  i. IgM – which is most potent activator of classical pathway
  ii. IgG – Which is 2nd most potent activator of classical pathway

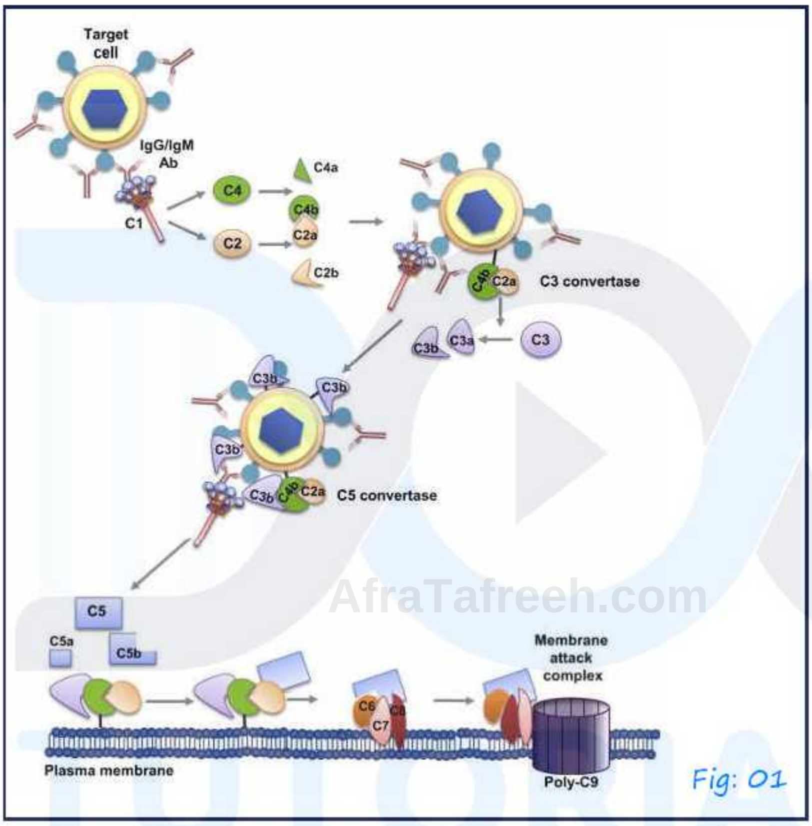

Target cell
IgG/IgM Ab
C1
C4
C2
C4a
C4b
C2a
C2b
C3 convertase
C3
C3a
C3b
C5 convertase
C5
C5a
C5b
Membrane attack complex
C6
C7
C8
Plasma membrane
Poly-C9

Fig: 01

- $C_1$ protein has 3 parts $C_{1q}$, C1r, C1s
  C1q-1 molecule is present
  C1r & C1s – 2 molecules each are present
- C1s – has esterase activity
- C14b2a → is called as $C_3$ convertase
- C14b2a3b → is called as $C_5$ convertase
- $C_5b$ It wont bind back to complex.
- Instead $C_5b$ binds to $C_6$, $C_7$, $C_8$ & multiple molecules of $C_9$ to form Membrane Attack Complex (MAC)

- Membrane attack complex inserts holes in the cell membrane of pathogen.
- This causes the cell death.

C3a
C4a – They are called as ANAPHYLATOXINS
C5a

- Receptors for these proteins are present on Mast cells & Basophils

When the protein ($C_3a/C_4a/C_5a$) binds to the respective receptor on mast cell.

↓

Degranulation of mast cells occurs

↓

Histamine is released

↓

- Causes smooth muscle contraction
- Increases vascular permeability
- It is an important mediator of anaphylaxis, inflammation.

- $C_3b$ – is a very good opsonin.

# COMPLEMENT PROTEINS – II

## Alternative pathways 00:01:00

- Activated/Trigged by activator molecule
- Activator molecular
  - LPS/Endotoxin
  - Chitin
  - Snake venoms
  - CRP
  - IgA
  - IgD
  - IgG4
- In serum most abundant complement protein is C3
- In the absence of any activator

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$C3 \xrightarrow[\text{Broken down}]{\text{Spontaneously}} C3a + C3b$ – ($C3b$ = Is highly reactive)

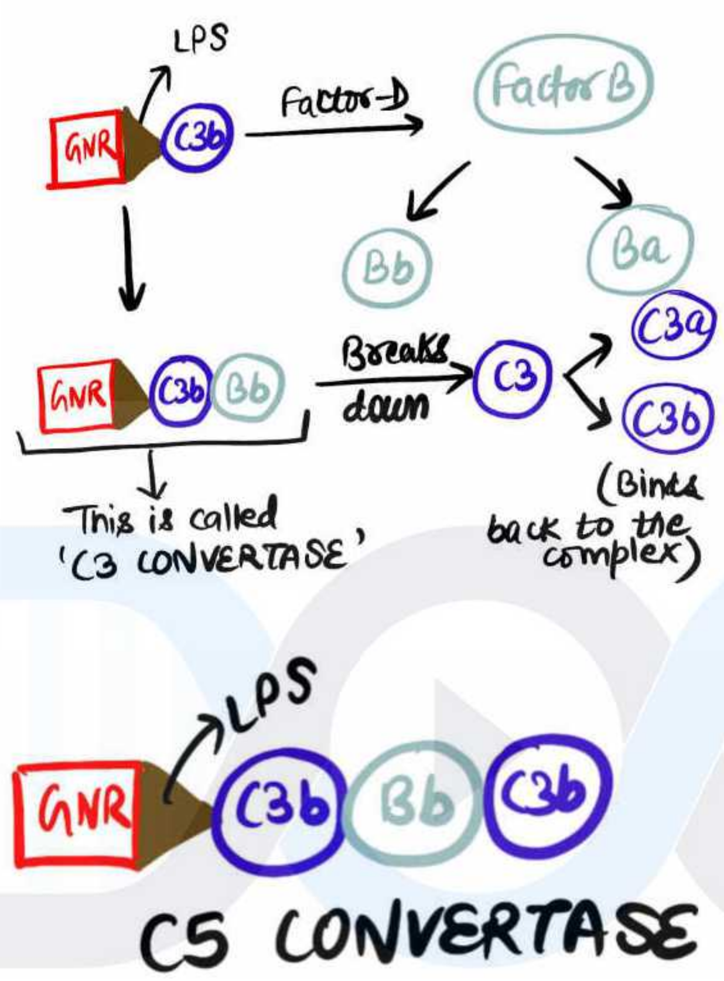
$$C3b + H_2O \rightarrow Ic3b$$

- If any activator is present
- e.g. Infection by G-ve bacterium

$$\begin{tikzcd}
1.\text{G-ve bacterial } C3b \arrow[d] & C3b \arrow[l] \\
\text{LPS} &
\end{tikzcd}$$

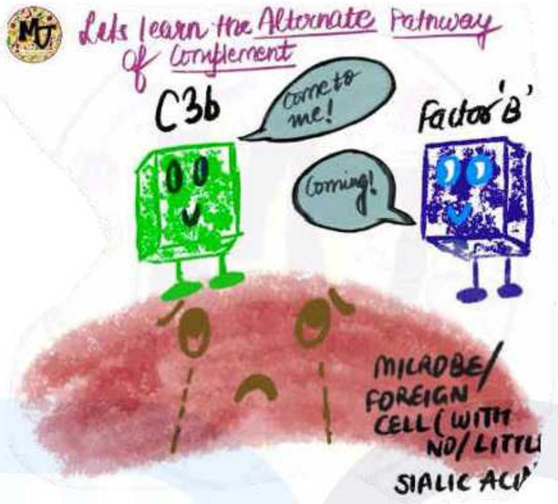
$$\begin{tikzcd}
2.\text{G-ve B } C3b \arrow[d] & C3 \arrow[l] \arrow[d] \\
\text{LRS} & C3a
\end{tikzcd}$$

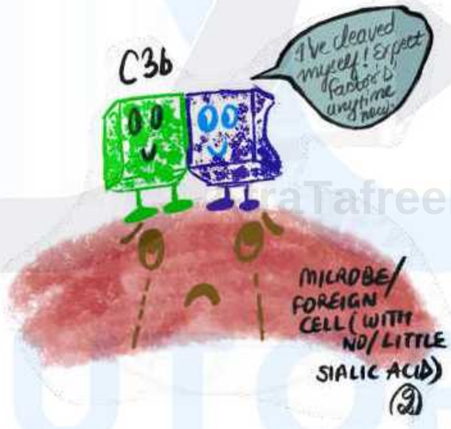
- This bacteria & C3b complex

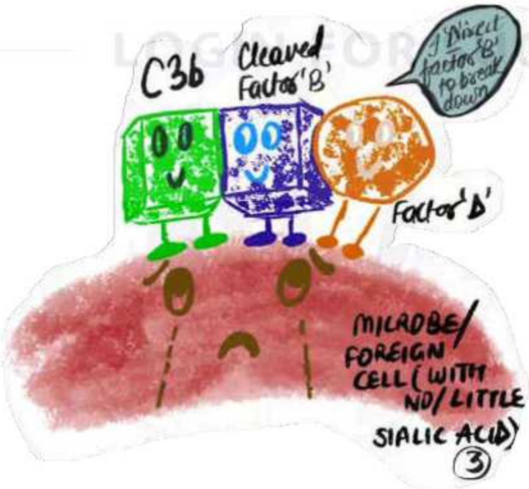

LPS
GNR
C3b
Factor-D
Factor B
Bb
Ba
GNR
C3b
Bb
Breaks down
C3
C3a
C3b
(Binds back to the complex)
This is called 'C3 CONVERTASE'
LPS
GNR
C3b
Bb
C3b
C5 CONVERTASE

- C5b Wont bind back to original complex but binds C6, C7, C8 & Multiple molecules of C9 to form membrane attack complex (MAC)
- MAC has
  - C5b
  - C6
  - C7
  - C8
  - C9 multiple molecules
- MAC can attack & kill both G+ and G-organism but G-organisms are easily attacked and killed more easily by MAC

- This is due the thin peptidyl can layer present in G- organism compared to the thick PG layer in G+ organisms


Lets learn the Alternate Pathway of Complement
C3b
Come to me!
Factor 'B'
Coming!
MICROBE/ FOREIGN CELL (WITH NO/LITTLE SIALIC ACID


C3b
I've cleaved myself! Expect factor 'D' anytime now.
MICROBE/ FOREIGN CELL (WITH NO/LITTLE SIALIC ACID)
②


C3b
Cleaved Factor 'B'
I Need factor 'B' to break down
Factor 'D'
MICROBE/ FOREIGN CELL (WITH NO/LITTLE SIALIC ACID)
③

IraTafreeh.com
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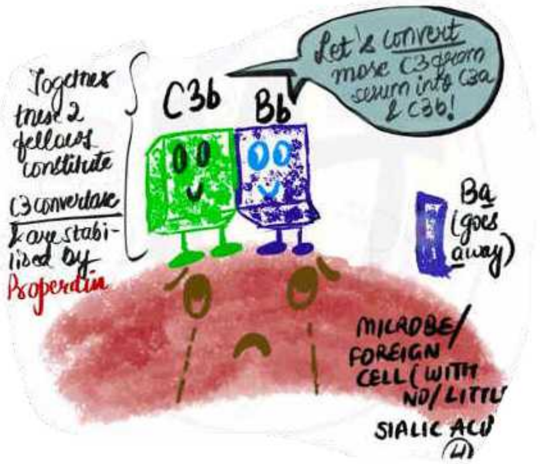
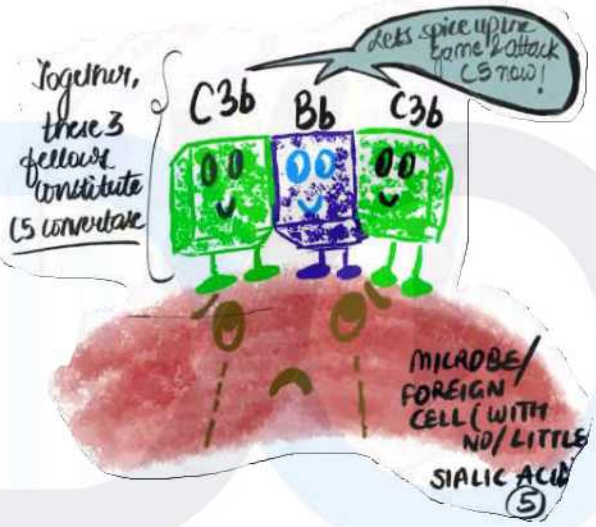
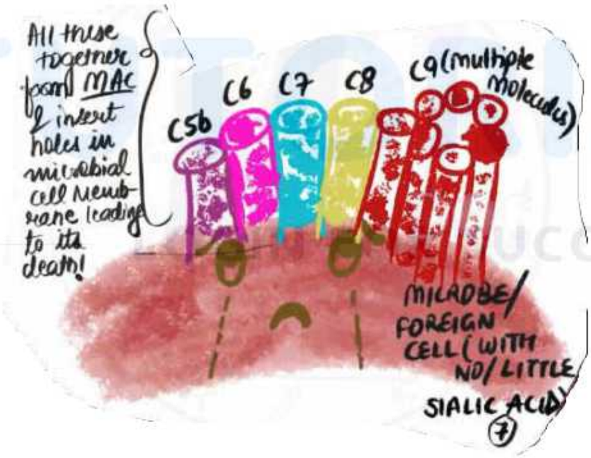
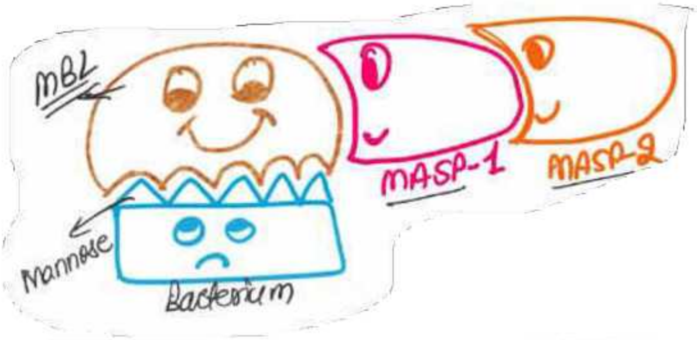

Together these 2 fellows constitute C3 convertase & are stabilised by Properdin
C3b
Bb
Let's convert more C3 from serum into C3a & C3b!
Ba (goes away)
MICROBE/ FOREIGN CELL (WITH NO/ LITTLE SIALIC ACID
(4)


Together, these 3 fellows constitute C5 convertase
C3b
Bb
C3b
Lets spice up the game & attack C5 now!
MICROBE/ FOREIGN CELL (WITH NO/ LITTLE SIALIC ACID
⑤

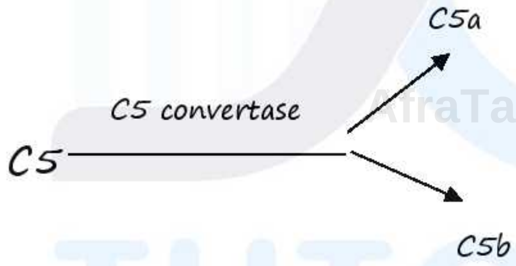

All these together form MAC & insert holes in microbial cell membrane leading to its death!
C5b
C6
C7
C8
C9 (multiple molecules)
MICROBE/ FOREIGN CELL (WITH NO/ LITTLE SIALIC ACID)
⑦

## Lectin pathways 00:30:10

- It is activated by a protein known as mannose binding lectin/MBL


MBL
Mannose
Bacterium
MASP-1
MASP-2

- MASP: MBL associated serine protease
- C5 convertase is formed


C5
C5 convertase
C5a
C5b

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- C5b want bind to original complex instead it forms MAC along with C6, C7, C8 & Multiple molecules of C9

## Deficiency of complement proteins can lead to some disorders 00:40:10

| Disorder of complement deficiency | Deficient complement Proteins |
|---|---|
| • Group – I: Autosomal recessive characterized by immune complex deposition in body i.e. immune complex mediated disease like SLE | • C1, C2, C4 are deficient |
| • Group II<br>Hereditary angioedema<br>Autosomal dominant | • C1 esterase inhibitor is deficient<br>• It is a regulatory protein<br>• Degrades C1s which has esterase activity<br>• Its deficiency leads to uninterrupted progression of classical pathway<br>• This leads to higher generation of C5a, C4a, C3a |

| | |
|---|---|
| | • These are anaphylatoxins |
| • Group III<br>Autosomal recessive | • C3 deficiency<br>• Due to deficiency of C3b – there is increased incidence of pyogenic/ suppurative infections |
| • Group IV<br>Autosomal recessive | • C5 –C8 (late complement proteins)<br>• Therefore ↓ In late complement proteins leads to ↓ MAC<br>• This leads to increased incidence of gram-negative infection specially Neisseria & Toxoplasma gondii |
| • Group V<br>Autosomal recessive | • C9 deficiency<br>Features are similar to group IV |

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## In alt pathway 00:55:28

- Factor – H + C3b → Factor – H, C3b

$$\downarrow \quad \text{Factor I}$$

Thus, C3b is destroyed & Alternate pathway can't proceed further where there is enough C3b which can bind microbial surface

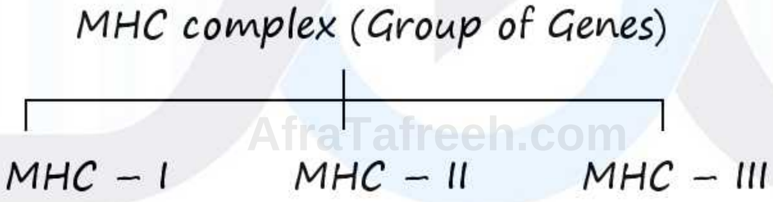
- DAF/Decay accelerating factor/CD55
- This is a GPI linked protein present on our cells (E.g.: RBCs & Endothelial cells)
- DAF – Breaks down C4b & C5b
- GPI: Glycose phosphatidyl inositol

# MHC COMPLEX & ANTIGEN PROCESSING

## MHC Proteins 00:00:12

- Major Histocompatibility Complex proteins.
- They are also known as Human Leukocyte Antigens (HLA) .
- Genes for MHC proteins are present on the short arm of chromosome – 6 (6p)
- MHC molecules are GLYCOPROTEINS .

## Categories of MHC complex 00:05:08



- MHC – III have no role in Antigen presentation.
- These MHC – III genes encode for some proteins like
  - Factor B
  - Factor D
  - $C_3$ Convertase
  - TNF $\nearrow \alpha$ $\searrow \beta$
  - 21 – steroid hydroxylase
  - HSP proteins
  - Properdin

## MHC – I genes:

- These genes are expressed in all nucleated cells & platelets

## MHC – II genes:

- These are expressed on antigen presenting cells.
  Eg: Dendritic cells – Best APCs
  Macrophages
  B – cells
- MHC – I proteins present Endogenous Ags (proteins) to $T_C$ cells (CD8+T cells)
- MHC – II proteins present exogenous Ags (proteins) to $T_H$ cells (CD4 + T cells)

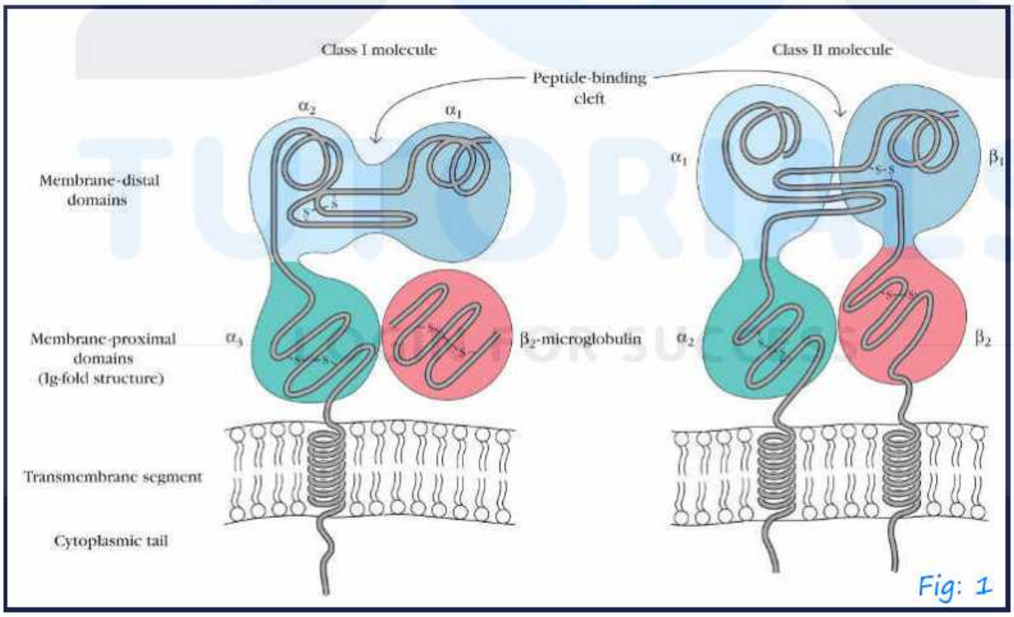

Class I molecule
Class II molecule
Peptide-binding cleft
α2
α1
α1
β1
Membrane-distal domains
Membrane-proximal domains (Ig-fold structure)
α3
β2-microglobulin
α2
β2
S–S
Transmembrane segment
Cytoplasmic tail

Fig: 1

- MHC – II have 3 loci

3 loci
DP
DQ
DR

- 3 loci which encode for 1 α chain & 1 β chain.
- Which form 3 different class – II MHC molecules

- Pocket/cleft of MHC – I molecule is small & accommodates 8-10 amino acids long peptide molecule.
- Pocket/cleft of MHC-II molecule is big & accommodates 13-18 amino acids long peptide molecule

| MHC – I | MHC – II |
|---|---|
| Peptide binding Cleft:<br>- It is formed by the groove between $\alpha_1$ & $\alpha_2$ domains | Bigger cleft (13-18 Amino acid long protein chain)<br>- formed by the groove between $\alpha_1$ & $\beta_1$ domains |

## Difference between Endogenous & Exogenous Proteins 00:30:00

**Endogenous proteins:**

- An Intracellular bacterium which is present inside the nucleated cell, multiples
  i. During this multiplication, the bacterium produces its own proteins known as Endogenous Proteins
    - One of the fragment/parts of endogenous protein is expressed on cell surface in the pocket of MHC – I molecule

## Exogenous proteins:

- When a bacterium enters the macrophage, the macrophage may not allow the multiplication of bacterium & break down the bacterium during phagocytosis into
  - i. Protein fragments
  - ii. Sugar fragments etc.
- Such protein fragments are known as Exogenous proteins
- This exogenous protein is expressed on the cell surface in the pocket of MHC – II molecule.

In extracellular organisms: Eg. S.aureus

- Enzymes, toxins are released onto the host cells.
- In these organisms, Ab-play on important role in immunity.

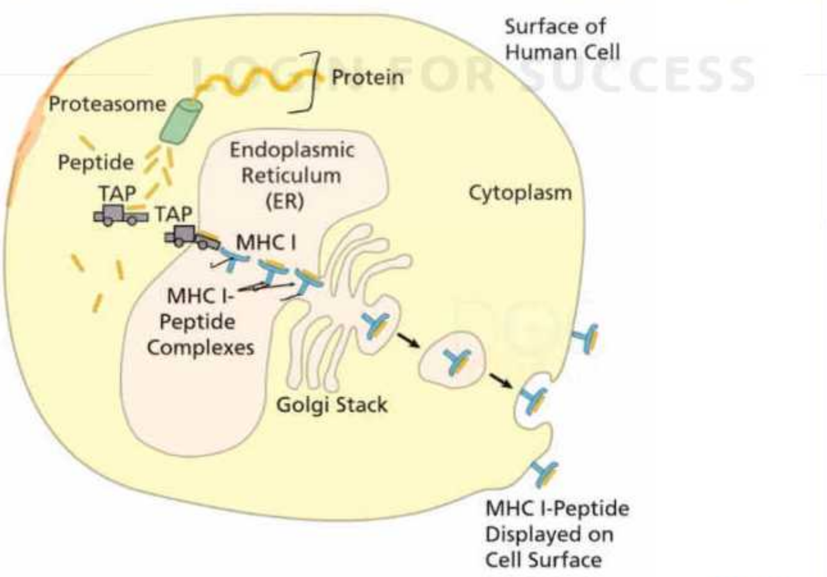

Surface of
Human Cell
Protein
Proteasome
Peptide
TAP
TAP
Endoplasmic
Reticulum
(ER)
Cytoplasm
MHC I
MHC I-
Peptide
Complexes
Golgi Stack
MHC I-Peptide
Displayed on
Cell Surface

Fig: 2

Ag-presentation in MHC- I molecule

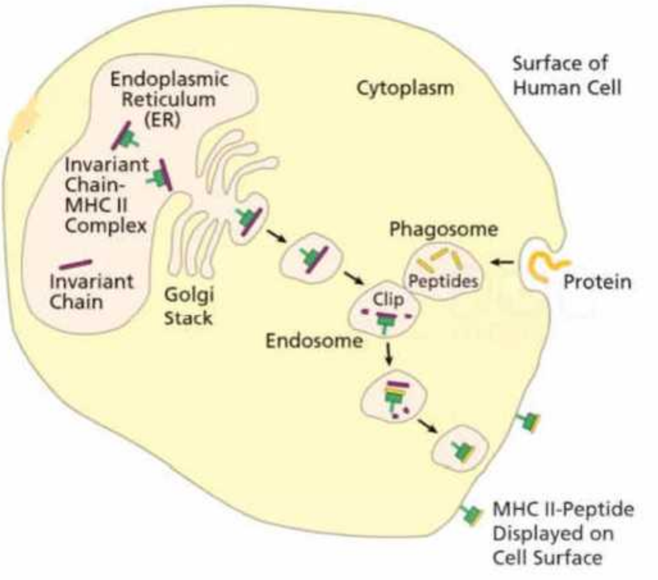

Endoplasmic Reticulum (ER)
Cytoplasm
Surface of Human Cell
Invariant Chain-MHC II Complex
Invariant Chain
Golgi Stack
Phagosome
Peptides
Protein
Clip
Endosome
MHC II-Peptide Displayed on Cell Surface

Fig: 3

Ag-presentation in MHC-II molecule

## Antigen Processing 00:57:35

| In MHC – I molecules | In MHC – II molecules |
|---|---|
| • Cytosolic pathway<br>• In this the endogenous proteins are fed into proteosome (protein – cutting apparatus)<br>• The broken peptides so formed are picked up by another protein known as Transporter Associated with Ag-Processing (TAP) | • Endosomal pathway<br>• In the ER, the pocket of MHC-II molecules is occupied by another protein called as "Invariant chain"<br>• This is to prevent the binding of endogenous peptides present in the ER<br>↓<br>• This complex of MHC-II & invariant chain is |

| | |
|---|---|
| • And then transported to ER where MHC-I proteins pick up the Endogenous peptide fragments & then finally via the Golgi apparatus are secreted into a vesicle<br>↓<br>Then expressed on the cell surface to be presented to $T_c$/CD8 + T cells – cytotoxic T cells | secreted into a vesicle via the Golgi apparatus<br>↓<br>Then the vesicle matures into Endosome<br>• This Endosome fuses with phagosome.<br>• This phagosome has the peptide fragments derived from microbe.<br>• The endosomal enzymes breakdown invariant chain into fragments One fragment namely CLIP protein occupies the pocket of MHC-II & facilitates the binding of peptide fragment derived from the microbe (present in the phagosome) to the pocket of MHC II, & CLIP itself leaves the pocket. |

# GENERATION OF TH1 CELLS & PATHOGENESIS OF HYPERSENSITIVITY REACTIONS & ROLE OF Tc CELLS & INTERPLAY BETWEEN Th & Tc CELLS

## Importance of MHC molecules 00:00:01

- MHC molecules are highly polymorphic
  - Poly – many
  - Morphic – morphology

| MHC-I | MHC-II |
|---|---|
| ↓ | ↓ |
| 3 loci | 3 loci |
| A | DP |
| B | DQ |
| C | DR |
| ↓ | ↓ |
| ≈ 1800 alleles | ≈ 800 alleles |

←→

As far as acquired immune response is concerned:

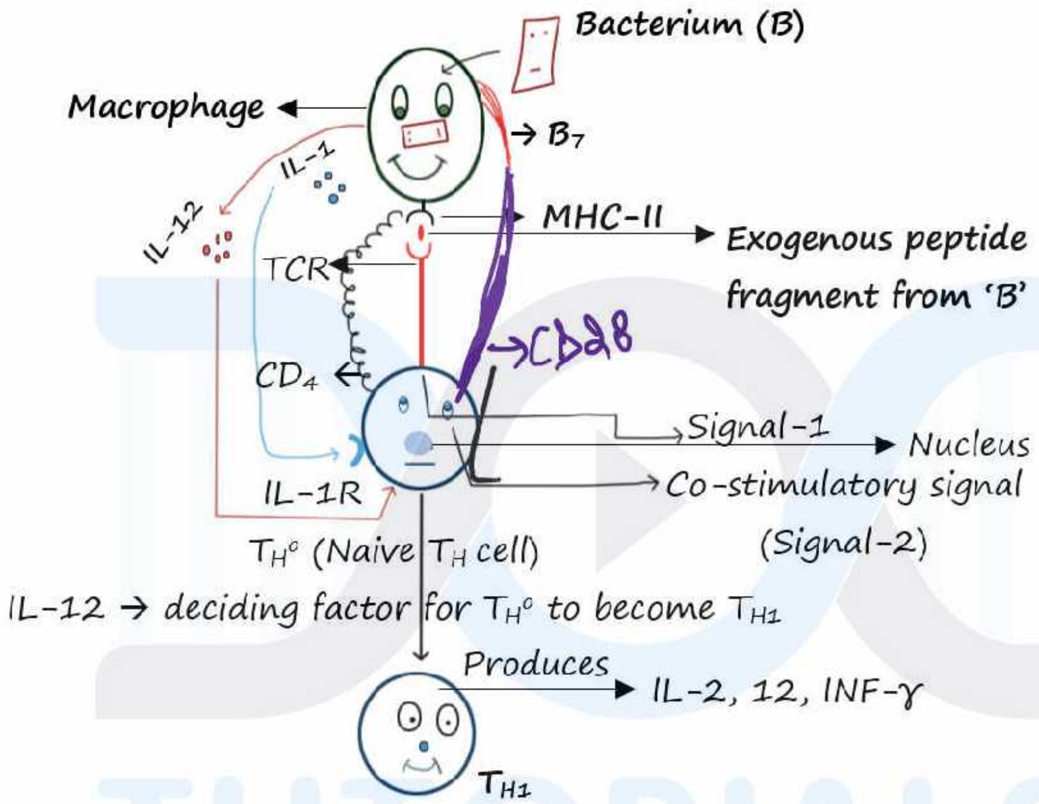
2 antigens

- Endogenous Ag
- Exogenous Ag

## Exogenous antigens 00:05:14

- Think of MHC-II ↔ $CD_4$

$$\begin{tikzcd} \text{MHC-II} \arrow[d, leftrightarrow] & CD_4 \arrow[d] \\ \text{APCs} & T_H \text{ cells} \end{tikzcd}$$


Bacterium (B)
Macrophage
$B_7$
IL-1
IL-12
MHC-II
Exogenous peptide fragment from 'B'
TCR
CD28
$CD_4$
Signal-1
Nucleus
Co-stimulatory signal (Signal-2)
IL-1R
$T_H^o$ (Naive $T_H$ cell)
Produces
IL-2, 12, INF-$\gamma$
$T_{H1}$

IL-12 → deciding factor for $T_H^o$ to become $T_{H1}$

- Note: 'B' doesn't multiply inside the macrophages but gets broken down by macrophages

## Clonal selection 00:25:45

- In the above case, one out of thousands of T-cells is selected, the one whose TCR can recognize the Ag brought by APC
- IL-2 = T-cell growth, factor (TCGF), helps in multiplication of $T_H$, $T_C$ cells (these cells have receptors for IL-2)

After clonal selection

↓ Next

IL-2 acts on $T_{H1}$ cells and causes its multiplication

(Autocrine action of IL-2)

## Clonal proliferation 00:27:20

- INF-$\gamma$ = Most potent activator of macrophages

Macrophage $\xrightarrow{INF-\gamma}$ Hyperactive

(Increase Exp of MHC & increased phagocytic potential, free radical formation)

## Hyperactive macrophages reach the site of infection 00:31:00

Hyperactive macrophages reach the site of infection

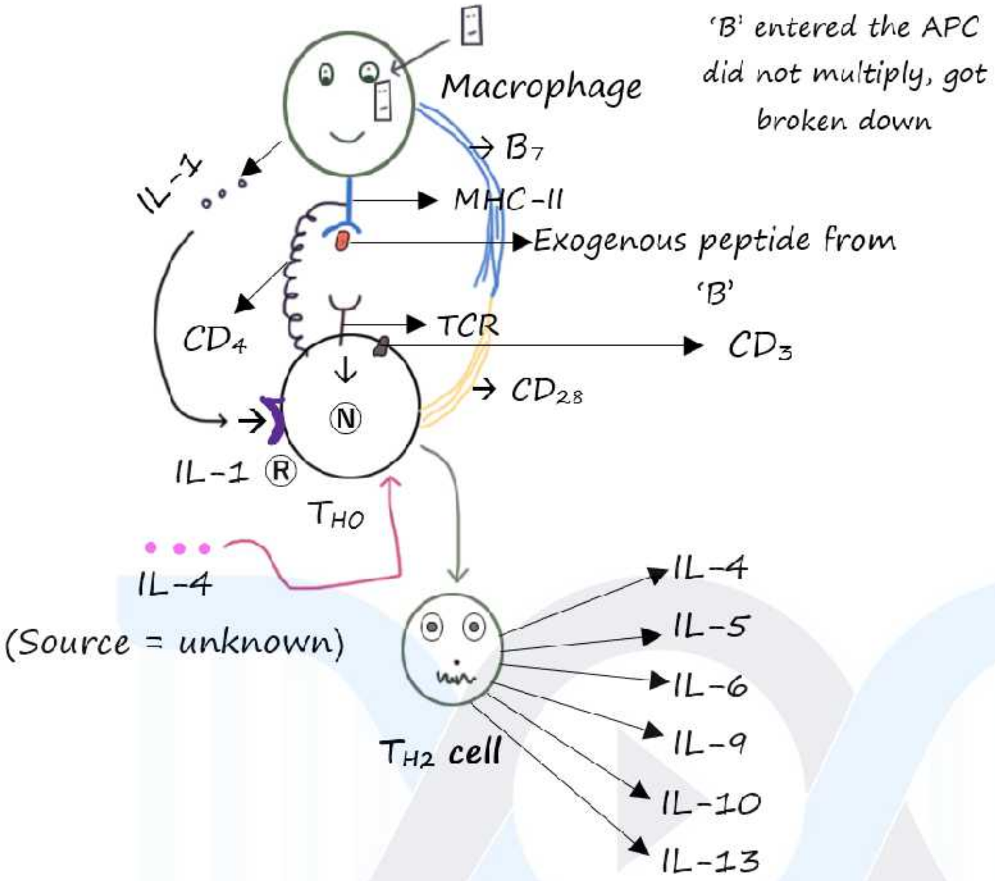
Clear the microbe successfully

Conclusion: CMI (Cell Mediated Immunity achieved)

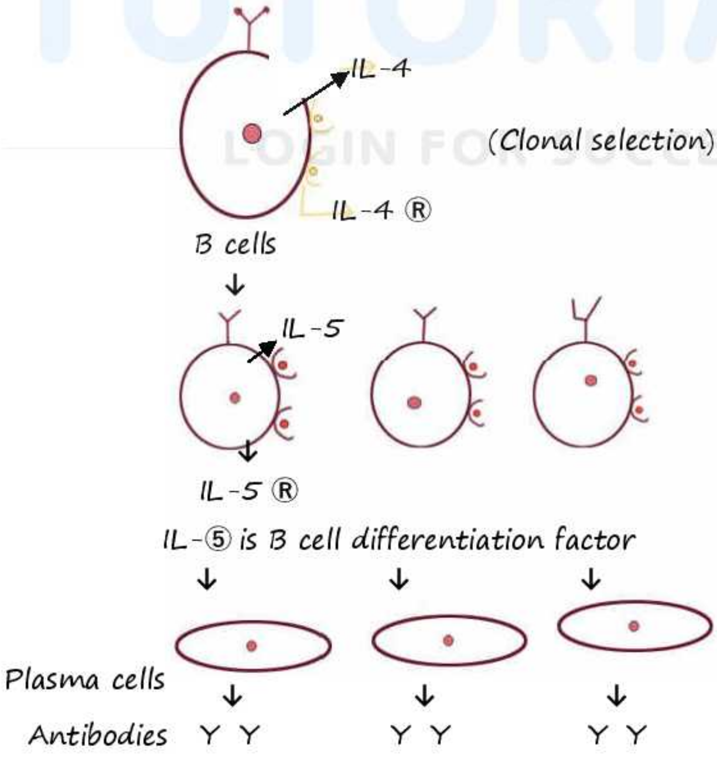
Tissue damage may also occur

↓

Hypersensitivity reaction Type-IV (No role of antibodies )


'B' entered the APC did not multiply, got broken down
Macrophage
IL-1
→ B7
MHC-II
Exogenous peptide from 'B'
CD4
TCR
CD3
→ CD28
N
IL-1 R
THO
IL-4
(Source = unknown)
TH2 cell
IL-4
IL-5
IL-6
IL-9
IL-10
IL-13

- IL-4,5 → act on 'B' cells


IL-4
(Clonal selection)
IL-4 R
B cells
IL-5
IL-5 R
IL-⑤ is B cell differentiation factor
Plasma cells
Antibodies

These antibodies will bind to more microbes

Ab

**Abs can eliminate microbe via**

i. Phagocytosis (opsonization)

ii. ADCC

iii. Complement activation

↓ Conclusion

Humoral immunity achieved

**Tissue damage may occur also**

↓

Type-I/II/III H/S-Ab mediated

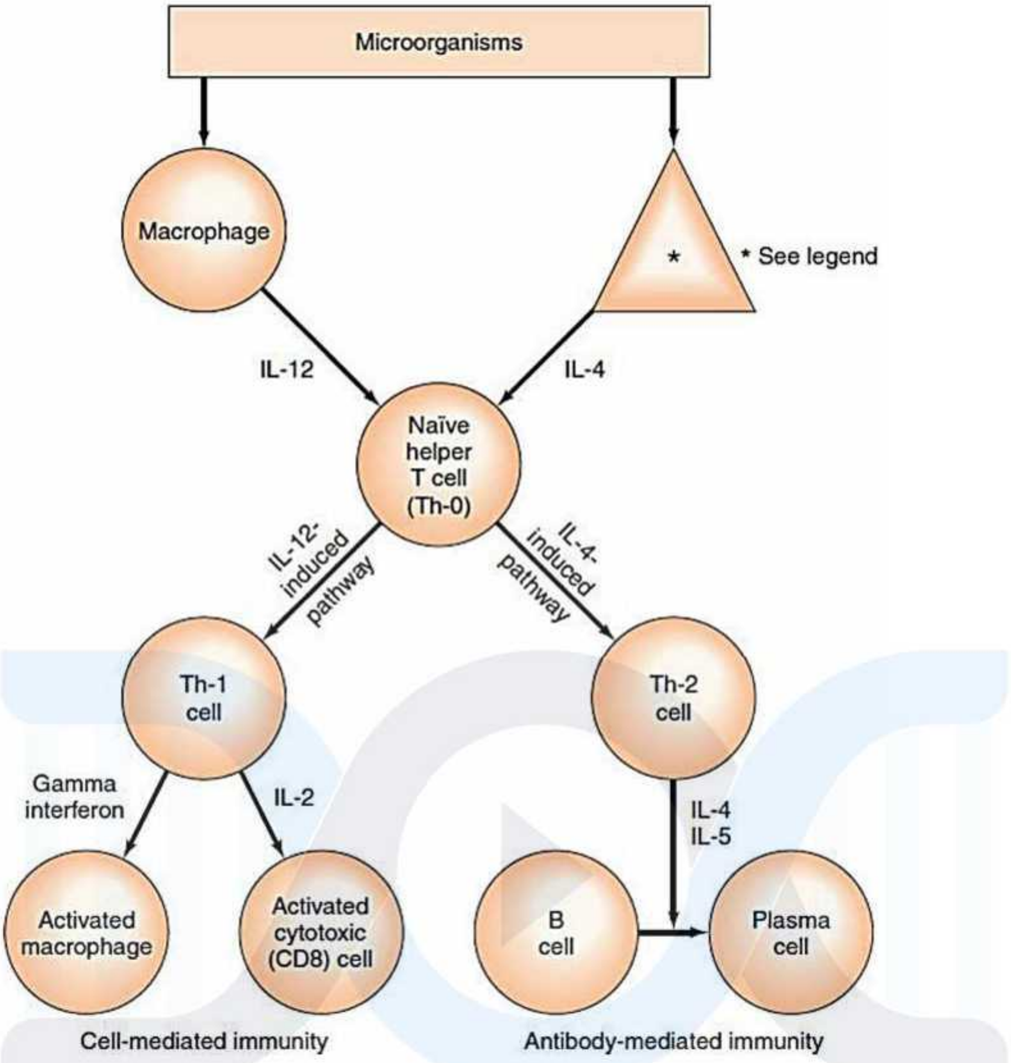
Phagocytosis of microbes by

↓

Macrophage
Or
Dendritic cell

Innate immunity → Destroy microbes and secrete cytokines

Acquired immunity → Present antigens to CD4-positive helper T cells

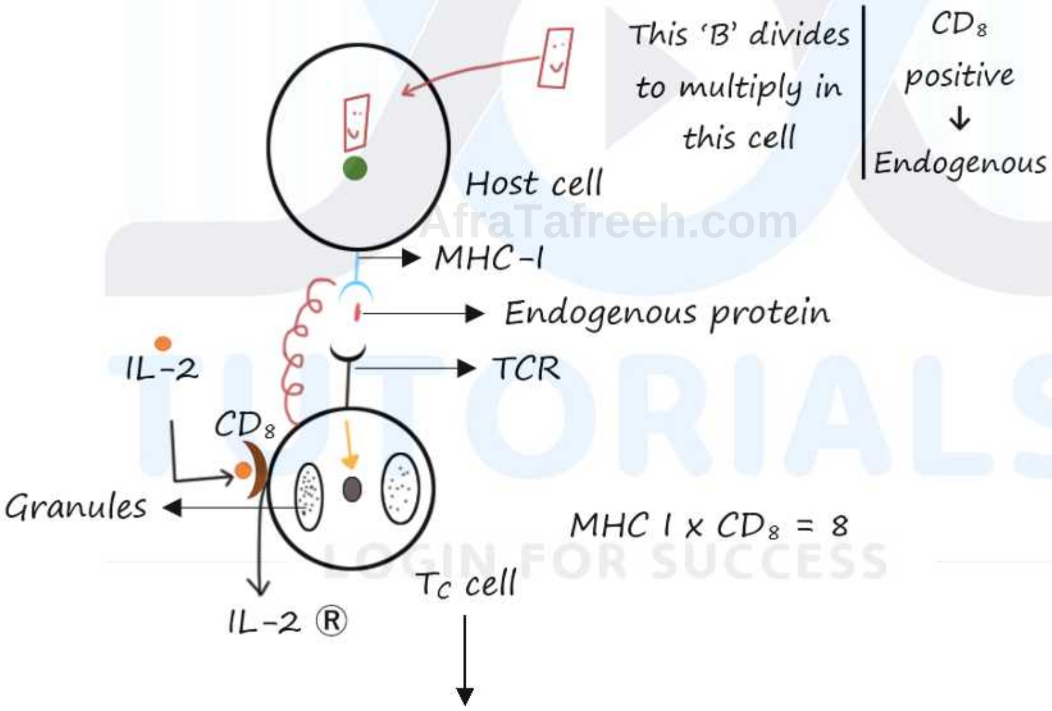

Microorganisms
Macrophage
*
* See legend
IL-12
IL-4
Naïve helper T cell (Th-0)
IL-12-induced pathway
IL-4-induced pathway
Th-1 cell
Th-2 cell
Gamma interferon
IL-2
IL-4
IL-5
Activated macrophage
Activated cytotoxic (CD8) cell
B cell
Plasma cell
Cell-mediated immunity
Antibody-mediated immunity

## Differences between $Th_1$ and $Th_2$ 00:54:54

| Property | $Th_1$ cells | $Th_2$ cells |
|---|---|---|
| Produces IL-2 and γ interferon | Yes | No |
| Produces IL-4, IL-5 and 10 | No | Yes |
| Enhances cell mediated immunity and delayed hypersensitivity | Yes | No |
| Enhanced Ab production | No | Yes |
| Stimulated by IL-12 | Yes | No |
| Stimulated by IL-4 | No | Yes |

## $T_C$/Cytotoxic cells – CMI 00:57:12

- Have cytoplasmic granules with contain
  - Perforins
  - Granzymes
- Have excellent activity against:
  - i. Tumor cells
  - ii. Virus-infected cells
- IL-2: T cell Growth factor
  - Produced by $T_{H1}$


This 'B' divides
to multiply in
this cell
$CD_8$
positive
↓
Endogenous
Host cell
MHC-I
Endogenous protein
TCR
IL-2
$CD_8$
Granules
MHC I x $CD_8$ = 8
$T_C$ cell
IL-2 ®

On getting fully activated: - Perforins and granzymes are released from the granules of $T_C$ cell and act on infected host cell and lyse the cell

# Difference between $T_C$ cells and $N_K$ cells

01:09:20

| $T_C$ cells | NK Cells |
|---|---|
| Acquired immunity<br>Kill those cells with show up regulation of MHC-I molecules | Innate immunity<br>Kill those cells with show down regulation or decreased expression of MHC-I molecules |

- Therefore, Tc cells are MHC-I restricted

↓

In the context of Ag presentation

- $T_H$/helper T-cells: are MHC-II restricted

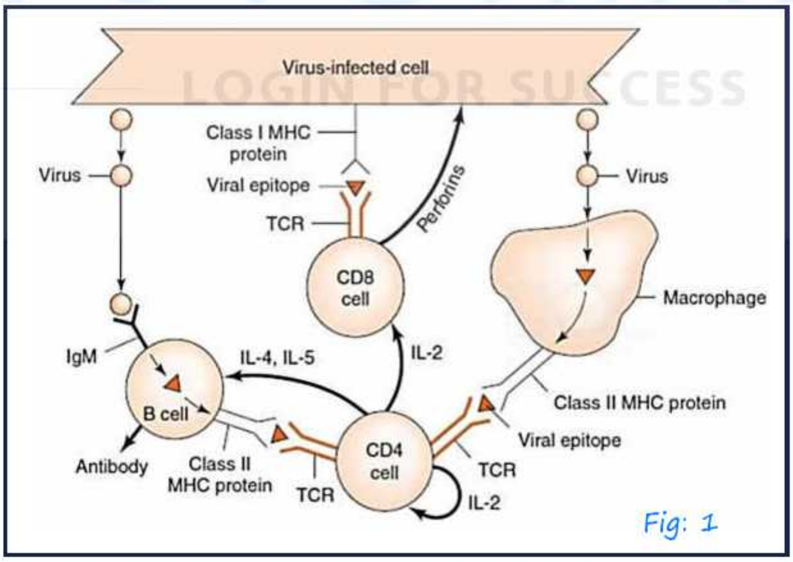

Virus-infected cell
Class I MHC protein
Viral epitope
TCR
Perforins
Virus
Virus
CD8 cell
Macrophage
IgM
IL-4, IL-5
IL-2
B cell
Class II MHC protein
Viral epitope
CD4 cell
TCR
Antibody
Class II MHC protein
TCR
IL-2

Fig: 1

## CTLA-4 protein: 01:30:42

Towards the later course of immune response

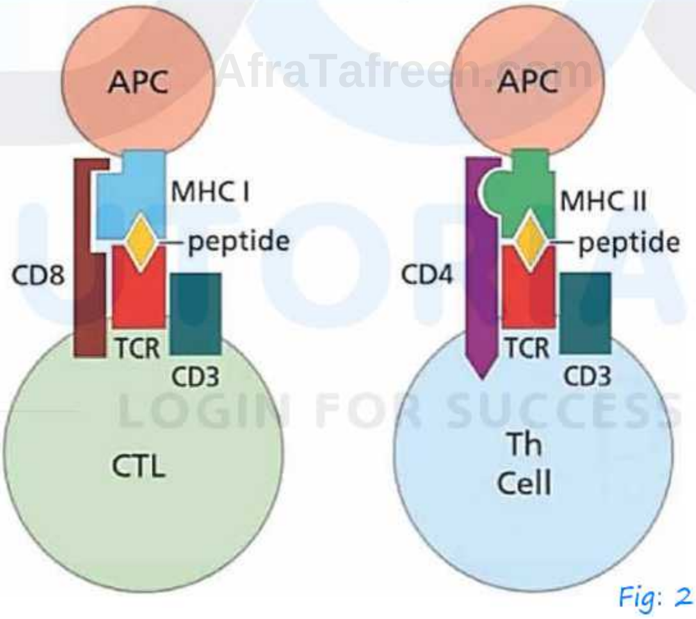
(Once the infection has been cleared, the cytoplasmic protein of $T_H$ cells known as CTLA-4(cytotoxic T-lymphocyte associated Ag-4) is expressed on $T_H$ cells' surface, displaces $CD_{28}$, itself interacts with $B_7$. CTLA-4-has 1000 times more affinity for $B_7$ than $CD_{28}$

↓

No costimulatory signal anymore

↓

$T_H$ response dampens


APC
APC
MHC I
peptide
CD8
TCR
CD3
CTL
MHC II
peptide
CD4
TCR
CD3
Th
Cell

AfraTafreen.com

Fig: 2

# TD – TI ANTIGENS

- TI–Ag: T–cell/Thymus independent Ag
- TD–Ag: T cell/Thymus dependent Ag

## Differences between TI – Ag and TD – Ag (Thymus – dependent Ag) 00:00:50

| TI – Ag | TD–Ag |
|---|---|
| They directly stimulate the B–cells | T – cells are needed to stimulate the immune response |
| No memory | Memory is present |
| There are no cytokines | Class switch is present, memory cells and class switch are due to cytokines produced by T – cells |
| Example: –<br>•Polysaccharide capsule of bacteria<br>•Bacterial flagella | Example: –<br>•Protein Ags |
| Most of the Ags are TD – Ags | |

## TI – Ag 00:05:31

- B – cells are activated when the antigens bind to BCR on B–cells (example: capsular antigens binding to BCR)

- Nonspecific signals and signals due to cross-linking of BCRs activate B-cells
- Activated B-cells produce IgM Abs against the Ag. These Abs are identical to the BCR
- These Abs are IgM – immediate / acute infection

## TD – Ag 00:05:31

- Example: proteins

B – cells carry out receptor- mediated endocytosis

- Due to this:
  - a)Proteins are processed and expressed on B-cell surface in the pocket of MHC – II
  - b)Recycling of BCRs back to the surface of B – cells

- When a protein antigen binds to BCR on B-cells, 3 signals are required for activation of B-cells.
  - i. 1st signal due to cross-linking of BCRs
  - ii. 2nd signal is due to interaction between CD40 molecule and CD40 -L (present on surface of T-cell)
  - iii. 3rd signal comes from activated T-cell in the form of cytokines ($T_H2$ cell)
- This concept is used in the development of conjugate vaccines – example: HIB vaccine
- The cytokines are released from T-cells (IL-4,5) which act on B-cell and produce IgG antibodies
- Therefore, in TD -Ag -IgM, IgG and memory B and T-cells are produced

# SUPER ANTIGEN

## Superantigens

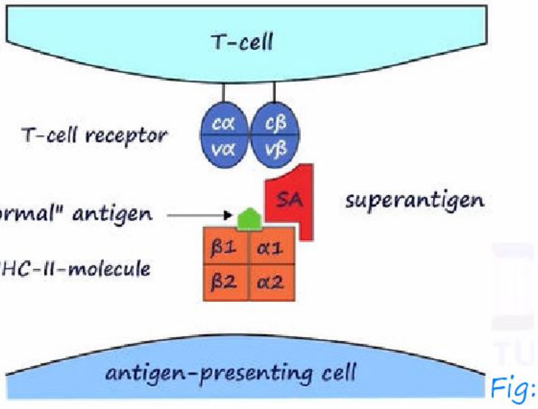

T-cell
T-cell receptor
cα
vα
cβ
vβ
SA
superantigen
"normal" antigen
MHC-II-molecule
β1
α1
β2
α2
antigen-presenting cell

Fig: 1

Antigen

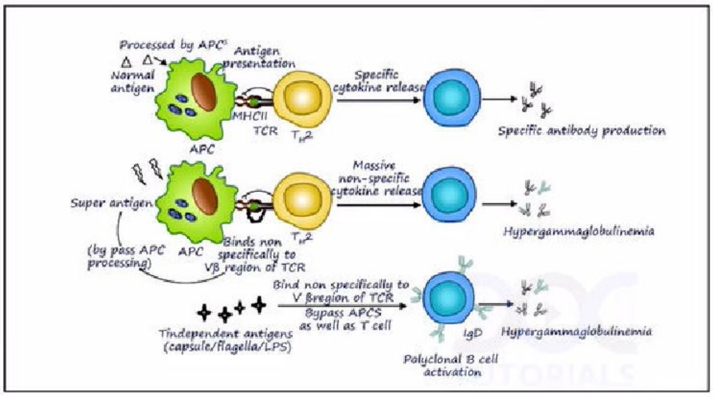
↓

Antigenic peptide

↓

Dendritic cells

- Super antigen binds at a different site & there is release of cytokines leading to massive release of B cells (globulins) leading to hyperglobulinemia


Processed by APC$^s$
Normal antigen
Antigen presentation
MHCII
TCR
$T_H2$
APC
Specific cytokine release
Specific antibody production
Super antigen
(by pass APC processing)
APC
Binds non specifically to Vβ region of TCR
$T_H2$
Massive non-specific cytokine release
Hypergammaglobulinemia
Bind non specifically to V βregion of TCR
Bypass APCS as well as T cell
Tindependent antigens (capsule/flagella/LPS)
IgD
Polyclonal B cell activation
Hypergammaglobulinemia

Mechanism of action of superantigens
Abbreviation: APCs, antigen-presenting cells; TCR, T cell receptor, MHC, major histocompatibility complex

Fig: 2

## Important Superantigens: 00:05:35

a. Bacterial

- Staphylococcal toxins
  - (i) Enterotoxin – Food poisoning
  - (ii) Toxic shock syndrome toxin (TSST)
    - Toxic shock syndrome
    - Scaled skin syndrome
- Streptococcal – Caused by SPE – A, C

b. Viruses

- (i) EBV superantigen
- (ii) HIV encoded superantigen
- (iii) CMV superantigen
- (iv) Rabies nucleocapsid

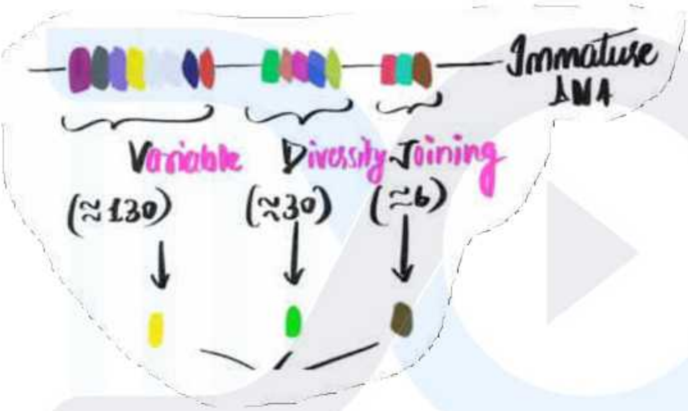
c. Fungi

- Malassezia furfur

# ANTIBODY DIVERSITY – I

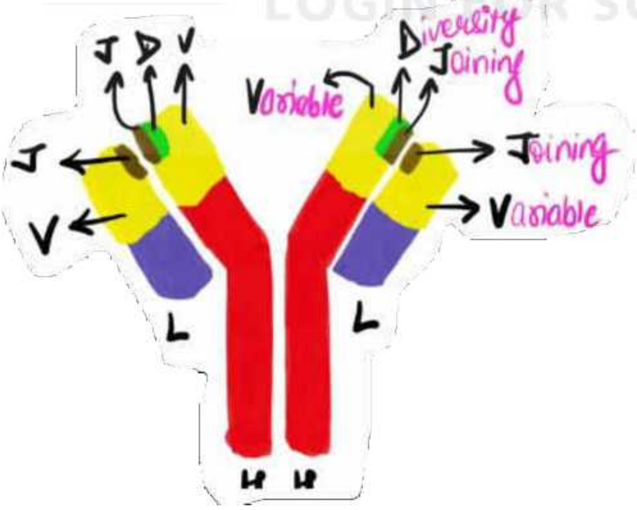
## Antibody Diversity 00:00:08

- Our immune system produces nearly 100million antibodies but B-cell have approximately 25000 genes only
- Chromosome 14 of B-cell

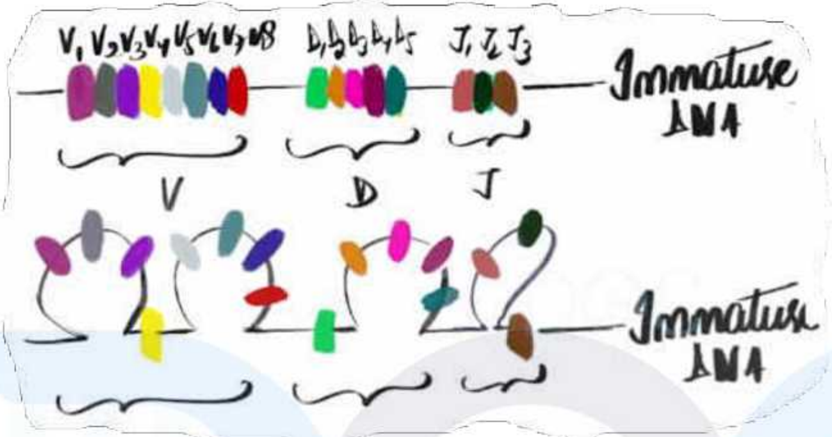

Immature DNA
Variable
Diversity
Joining
(≈130)
(≈30)
(≈6)

- Tonegawa theory: Appliable for all cells except B-cells
- Ab molecule

DOT TUTORIALS
LOGIN FOR SUCCESS

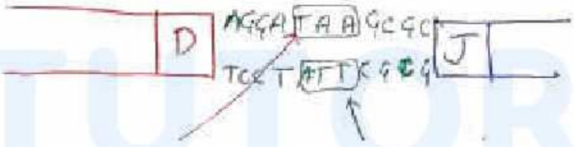

J
D
V
J
V
L
Variable
Diversity
Joining
Joining
Variable
L
H
H

- Recombinase enzyme – Responsible for gene rearrangement
- Gene rearrangement is responsible for B-cell diversity


V1 V2 V3 V4 V5 V6 V7 V8
D1 D2 D3 D4 D5
J1 J2 J3
Immature DNA
V
D
J
Immature DNA

## Terminal deoxyribonucleotide transferase (TDT)

- This enzyme adds "N" Nucleotideyl into chromosome & is responsible of B cell diversity


D
AGGA TAA GCGC
TCCT ATT CGCG
J

TDT adds N-Nucleotides

Repair enzymes adds complementary nucleotides

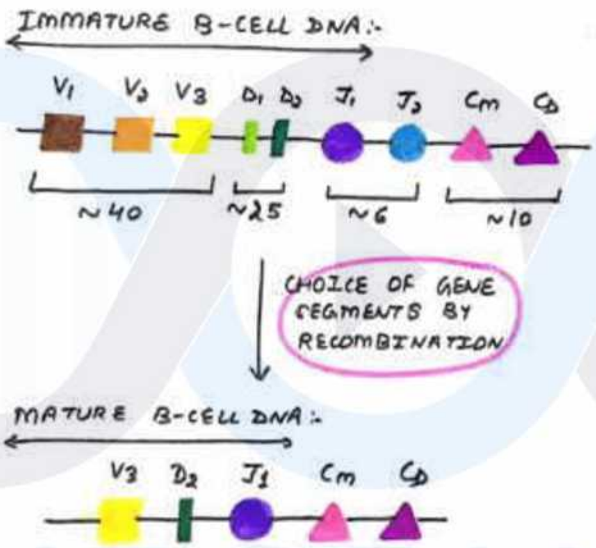
## Antibody diversity

1. Gene rearrangement because of recombinase enzyme
2. Terminal deoxyribonucleotidic enzyme (TDT)
   - TDT cause addition of N-Nucleotides
   - Recombinase enzyme (RAG): Picks up 1V, 1D & 1J segment during B-cell development
   - This occurs on chromosome – 14 (H Chain)

- L-chains: -
- K (Chromosome – 2)
- λ (Chromosome – 22)

- These have V, J segments & not D segment
- For the formation "L" chains same

1. Gene rearrangement
2. N-Nucleotide addition occurs


IMMATURE B-CELL DNA:-
$V_1$
$V_2$
$V_3$
$D_1$
$D_2$
$J_1$
$J_2$
Cm
Cδ
~40
~25
~6
~10
CHOICE OF GENE SEGMENTS BY RECOMBINATION
MATURE B-CELL DNA:-
$V_3$
$D_2$
$J_1$
Cm
Cδ

Q. The antigen binding site of an Ab is formed primary by

a. The constant region of H & L chains

b. The hypervariable regions of H & L-chains

c. The hypervariable regions of H-chains

d. The variable regions of H chains

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Answer: B

Bonds formed between Ag & Ab:

- Hydrophobic interactions
- Hydrogen bonds

- Ionic bonds/Electrostatic forces
- Vander wall forces
- The Ag – Ab reaction is firm but reversible
- No covalent bonds are formed between Ag & Ab

Q. The class of immunoglobin present in highest concentration in the blood of a human new-born is:

a. IgG – Natural passive immunity
b. IgM
c. IgA
d. IgD

Q. Cytotoxic T-cells induction with virus A will kill target cells

a. From the same host infected with a different virus
b. Infected by virus A & Identical at class I MHC loci of the cytotoxic T-cells
c. Infected by virus A & Identical at class II MHC loci of the cytotoxic T-cells
d. Infected with a different virus & Identical class I MHC loci of the cytotoxic cells

Answer: b

# TYPE – I HYPERSENSITIVITY REACTION

## Hypersensitivity reaction 00:00:10

- Any immune response which causes tissue damage is known as hypersensitivity reaction
- 4 types
  - Type – I ⎤
  - Type – II ⎬ Ab–mediated
  - Type – III ⎦
  - Type – IV – cell mediated

## Type – I / Immediate Hypersensitivity reaction 00:02:40

- Also known as Anaphylaxis
- Ab is IgE, it is fixed to mast cells and basophils
- No complement activation in type – I reaction
- Ag is free in circulation

  When Ag– enters the body for the first time

  ↓

  $T_H2$ cells are formed
- Atopy: is genetic predisposition to produce high levels of IgE Ab, because of excess production of IL–4
  - IL – 4 is involved in class switching of IgE
  - Atopy runs in families
- Excess IgE formation against common/innocuous environmental Ags occurs

- After the entry of Ag, IgE is formed against "allergen". This IgE goes and binds to receptors on mast cells and basophils
- On second time entry of same Ag into the body, Ag causes crosslinking of IgE receptors on mast cells and basophils
- Degranulation leads to production of histamine:
  Histamine – causes smooth muscle contraction
  - vasodilatation leading to edema
- Histamine is a primary / preformed mediator
  - They are present in cytoplasmic granules of mast cells and basophils

  Examples of preformed mediators
    1. Histamine
    2. Proteases – cleave complement proteins (C5a-a chemotactic agent for neutrophils)
    3. Neutrophil Chemotactic Factor (NCF)
    4. Eosinophil Chemotactic Factor (ECF)
    5. Heparin (not important in humans)
- These preformed mediators are responsible for immediate clinical features of Type I H/S reaction
- Delayed clinical features in type – I H/S reaction are due to the synthesis of secondary mediators:
  - Leukotrienes
  - Prostaglandins
  - Platelet activating factor (PAF)

- Leukotrienes are Slow Reacting Substance of Anaphylaxis (SRS-A)
- LTB4 – is a bronchoconstrictor
- Hygiene hypothesis – explains allergy

**Egs of type – I hypersensitivity reaction are:**

- Anaphylaxis
- Asthma
- Angioedema
- Acute urticaria (wheal and flare reaction)
- Allergic rhinitis (Hay fever)
- Allergy to food – peanuts, beans, egg proteins

# TYPE - II HYPERSENSITIVITY REACTION

## Type – II hypersensitivity reaction 00:00:09

- Also known as cytotoxic H/S reaction
- Ag is fixed on to a cell
- Abs are free:

  IgG or IgM
- Complement activation is present
- Once Ag – Ab complex is formed, the complex is eliminated by:
  - ADCC (Ab dependent cell mediated cytotoxicity)
  - Opsonization
  - Complement activation can form:

    a. MAC

    b. C3b

### In Myasthenia gravis:

- Ab to Ach-receptors is formed
- Therefore, Ach can not bind to Ach- receptors
- At NMJ, there is no local potential generated
- Therefore no action potential
- Thus muscle weakness develops

### In Graves disease:

- Ab against TSH receptor is formed

- These are Long-Acting Thyroid Stimulator Ab (LATS)
- These Abs are not under the negative feedback from pituitary gland

  Therefore, there is continuous stimulation of TSH receptor which leads to increased levels of T3, T4 hormones

## Egs of Type – II H/S reactions

i. Cell lysis / loss in the blood:
   - Hemolytic anaemia of newborn
   - Autoimmune hemolytic anemia
   - Blood transfusion reaction
   - Idiopathic thrombocytopenic purpura

ii. Cell lesion at local level
   - Good pasture's syndrome
   - Pernicious anemia
   - Pemphigus vulgaris
   - Acute rheumatic fever
   - Hyperacute graft rejection

## Cell's loss/gain of function:

- Myasthenia grains (Due to anti Ach ⊖R Ab)
- Type II diabetes mellitus
- Graves disease (due to anti ⊖TSH R Ab)

# TYPE – III HYPERSENSITIVITY REACTION

## Type –III Hypersensitivity reaction: 00:00:09

- Also known as immune complex mediated H/S reaction
- Both Ag and Ab are free
- Both Ag and Ab combine to form freely circulating complexes in blood leading to systemic reaction
- Big Ag and Ab complexes precipitate in tissues where they are formed
- Ab – can be IgM, IgG
- Complement activation is present

### Egs of type –III H/S reaction:

- SPARE farmers
  - Serum sickness
  - Systemic lupus erythematous
  - Post – streptococcal glomerulonephritis
  - Polyarteritis nodosa
  - Arthus reaction
  - Rheumatoid arthritis (type – IV > Type – III)
  - Erythema nodosum leprosum
  - Farmer's lung

# TYPE IV HYPERSENSITIVITY REACTION

## Type IV hypersensitivity reaction 00:00:11

a. Sensitization phase:

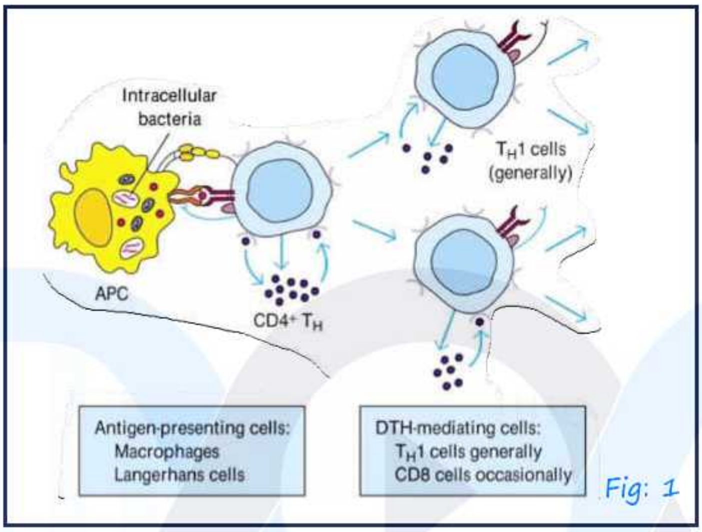
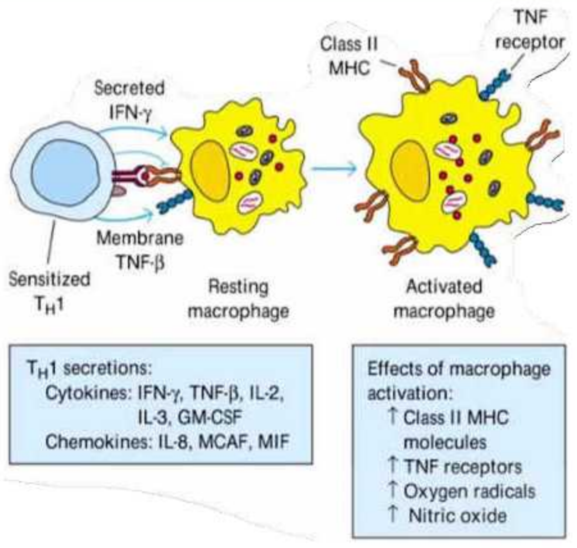

Intracellular bacteria
APC
CD4+ $T_H$
$T_H1$ cells (generally)
Antigen-presenting cells:
Macrophages
Langerhans cells
DTH-mediating cells:
$T_H1$ cells generally
CD8 cells occasionally

Fig: 1

- In some cases of Type-IV hypersensitivity reactions, Tc-cells also take part
- IFN-gamma – most potent activator of macrophages
  - Epithelioid cell → Macrophage with epithelial cell like configuration which has lost the phagocytic ability
- Granuloma formation = important feature of Type-IV hypersensitivity reaction


Class II
MHC
TNF
receptor
Secreted
IFN-γ
Membrane
TNF-β
Sensitized
$T_H1$
Resting
macrophage
Activated
macrophage
$T_H1$ secretions:
Cytokines: IFN-γ, TNF-β, IL-2, IL-3, GM-CSF
Chemokines: IL-8, MCAF, MIF
Effects of macrophage activation:
↑ Class II MHC molecules
↑ TNF receptors
↑ Oxygen radicals
↑ Nitric oxide

Effector phase Fig: 2

- Granuloma formation occurs in the effector phase

**Type-IV hypersensitivity reaction also known as delayed hypersensitivity reaction**

- It takes 48-72 hours to manifest

## Examples of Type IV hypersensitivity 00:10:09

- Multiple sclerosis
- Contact dermatitis (Poison Ivy)
- Insect bites
- Tuberculin test & transplant rejection
- Insulin dependent (Type-1) diabetes mellitus
- Guillain Barre syndrome (GBS)

# ANTIGEN ANTIBODY REACTIONS PART - I

## Antigen Antibody Reactions 00:00:16

- These occur at optimum concentration, pH, temperature and electro lytes
- Bonds between Ag and Ab are:
  - Hydrogen bonds
  - Hydrophobic interactions
  - Ionic bonds/electrostatic forces
  - Vander waal forces
- Ag -Ab reactions play an important role in serology

## Types of Antigen Antibody Reactions 00:03:01

- Based on conventional classification

  i. Precipitation reaction: Soluble Ag + Ab

  ii. Agglutination reaction: Particulate Ag + Ab

## Precipitation Reactions 00:04:20

- Reaction between soluble Ag and its corresponding antibody
- Less commonly done

## I. Precipitation Reactions in liquid medium 00:04:26

1.Ring test:

- In a narrow tube, antiserum is taken on which a layer of Ag-solution is added
- At the junction of these two, a precipitate ring is formed

  Example:

  - Streptococcal grouping by Lancefield technique
  - Ascoli's thermoprecipitin test for anthrax
- Precipitate ring is formed at the zone of optimum concentration of Ag and Ab known as zone of equivalence

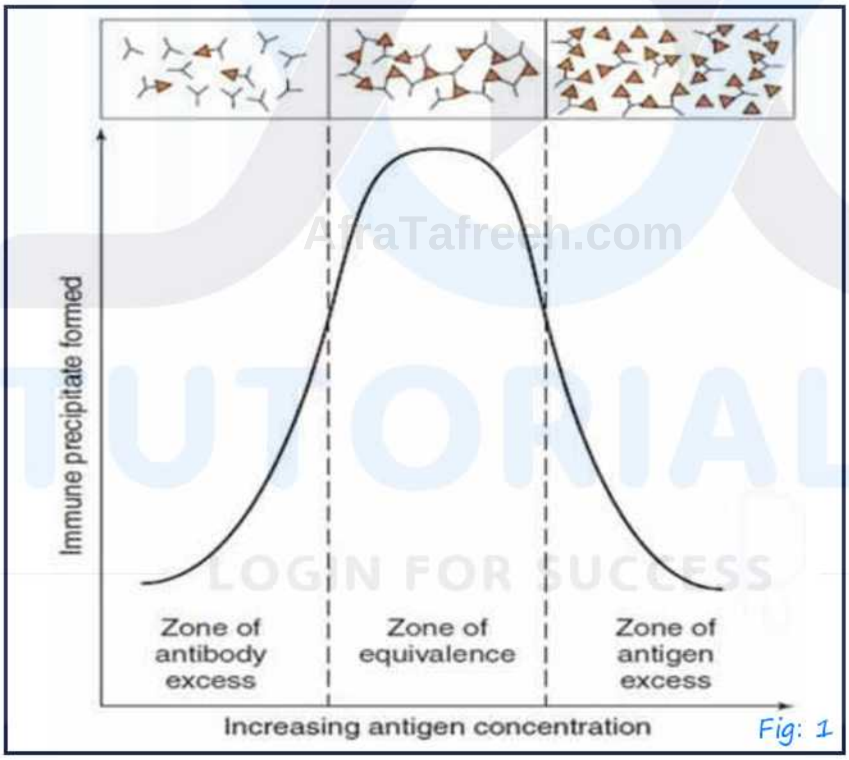


Fig: 1

- At the zone of equivalence, a lattice is formed – Marrack's lattice hypothesis

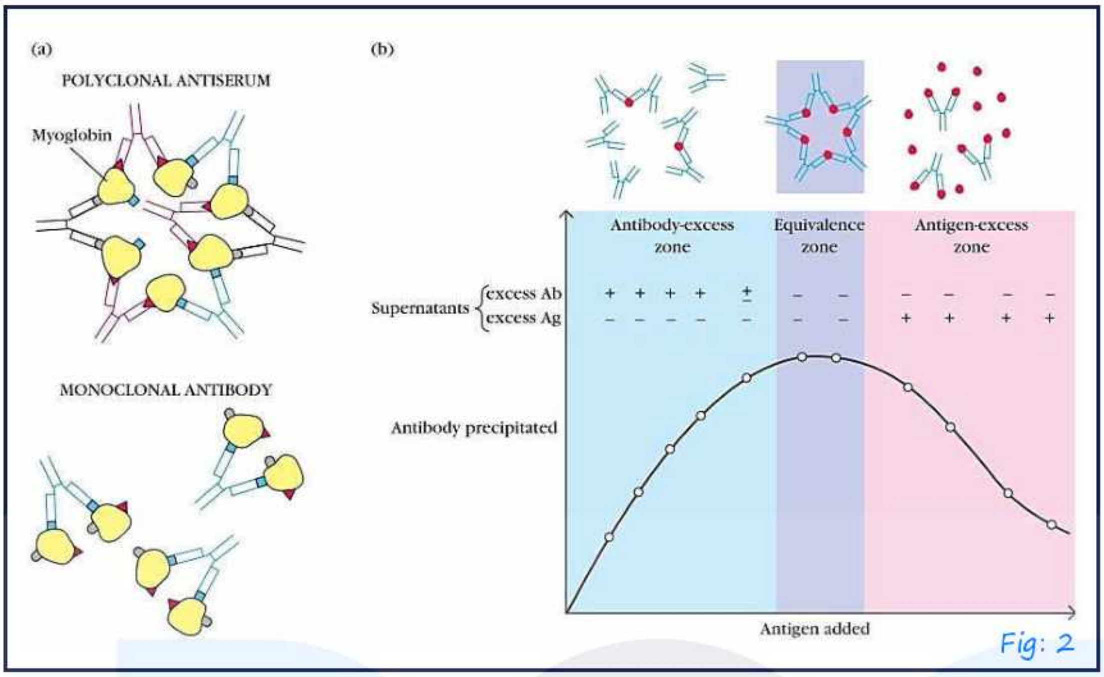

(a)
POLYCLONAL ANTISERUM
Myoglobin
MONOCLONAL ANTIBODY
(b)
Antibody-excess zone
Equivalence zone
Antigen-excess zone
Supernatants
excess Ab
excess Ag
+ + + + ±
– – – – –
– –
– –
– – – –
+ + + +
Antibody precipitated
Antigen added

Fig: 2

2.Flocculation test/reaction:

- A type of precipitation reaction
- Ag -Ab complex floats

  i. When a drop of Ag is mixed with patient's serum

  ↓

  Then a precipitate forms which remains suspended as floccules

  ii. This test can be done on a
    - Slide: slide flocculation test

      Example: VDRL and RPR
    - Test tube: tube flocculation test

      Example: Kahn Test

## II. Precipitation in gel/Immunodiffusion 00:15:15

- In this 1% soft agarose gel is incorporated

## Advantages:

- Visible 'Bands' are formed
  - These bands can be preserved for a longer time
- It can differentiate different antigens in a mixture
  - This is because each Ag will form a separate band after reacting with its specific Ab

## Principles:

1. Diffusion
   a) If only Ag is moving – Single diffusion
   b) If both Ag and Ab move: Double diffusion
2. Dimension
   a) If reaction occurs in test tube: one dimension
   b) If reaction occurs on a slide: two dimensions

## Four types of immune diffusion reactions in Gel:

1) Single diffusion in one-dimension:

- Also known as Oudin procedure

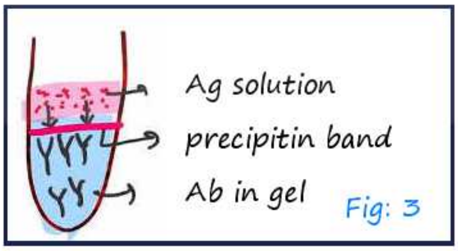


Fig: 3

2) Double diffusion in one-dimension

- Also known as Oakley fulthorpe procedure

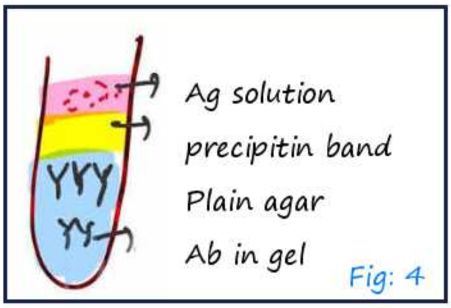

Ag solution
precipitin band
Plain agar
Ab in gel

Fig: 4

3) Single diffusion in two dimensions/ Radial immune diffusion

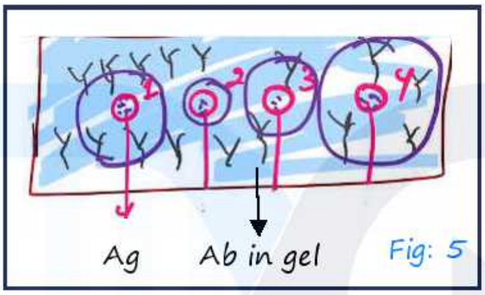

1
2
3
4
Ag
Ab in gel

Fig: 5

Rings of precipitation are formed

- Diameter of ring ∝ Ag concentration in serum of the patient

4) Double diffusion in two dimensions / uchterlony procedure

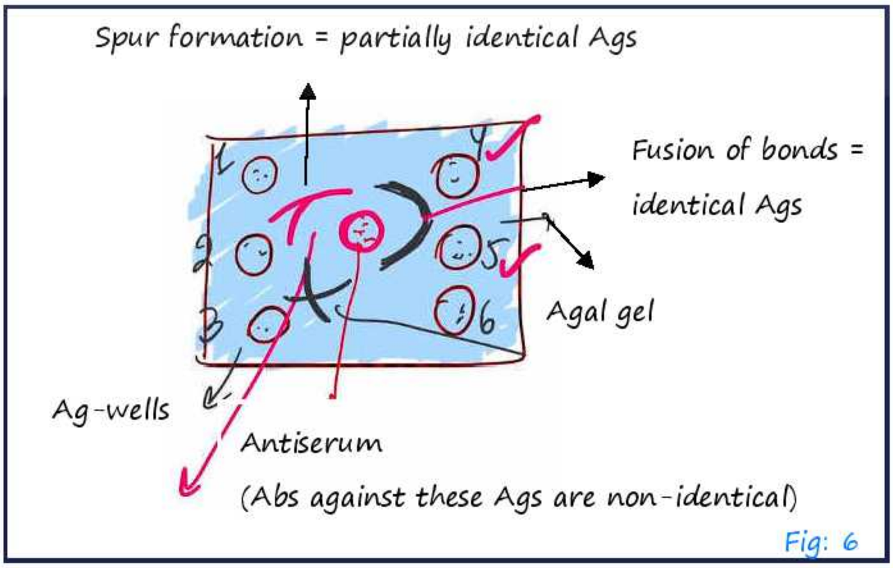

Spur formation = partially identical Ags
Fusion of bonds = identical Ags
1
2
3
4
5
6
Agal gel
Ag-wells
Antiserum
(Abs against these Ags are non-identical)

Fig: 6

**Example:**

- Elek's gel precipitation test: for C. diphtheriae
- Eiken's test: To detect 'LT' of ETEC

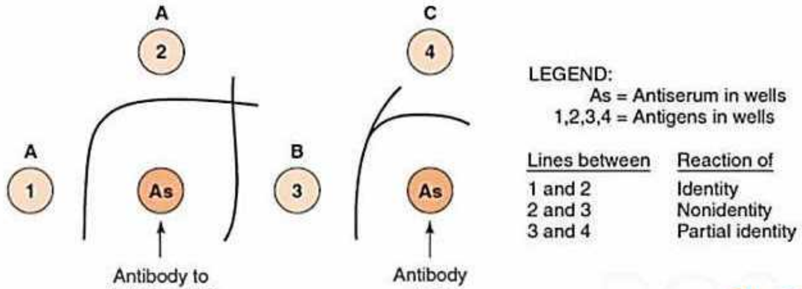

A
2
C
4
A
1
As
B
3
As
Antibody to both A and B
Antibody to B
LEGEND:
As = Antiserum in wells
1,2,3,4 = Antigens in wells
Lines between
Reaction of
1 and 2
Identity
2 and 3
Nonidentity
3 and 4
Partial identity

Fig: 7

- Precipitation in gel can be speeded up in the presence of electric current

  Example: -

  i. Electro immune diffusion (EID)

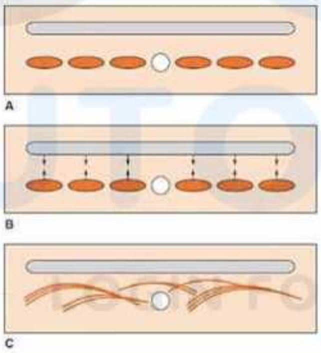
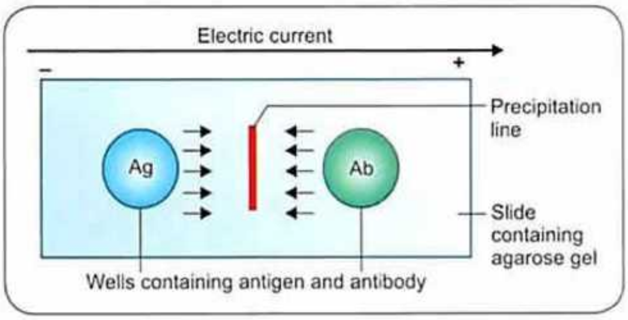


Fig: 8

DOC TUTORIALS LOGIC FOR SUCCESS

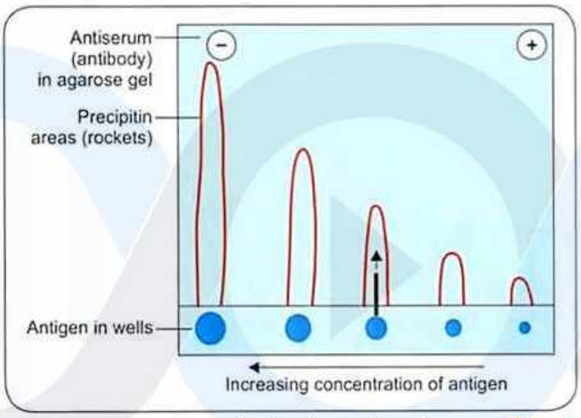
ii. Counter current immune electrophoresis (CIEP)

- Faster
- Takes 30 minutes
- More sensitive than EID
- Used to detect α – Fetoprotein in serum and capsular Ags of Cryptococcus and Meningococcus


Electric current
–
+
Precipitation line
Ag
Ab
Slide containing agarose gel
Wells containing antigen and antibody

Countercurrent immunoelectrophoresis Fig: 9

iii. Rocket electrophoresis:


Antiserum (antibody) in agarose gel
Precipitin areas (rockets)
Antigen in wells
−
+
Increasing concentration of antigen

Rocket electrophoresis Fig: 9

- It is done for quantitative estimation of Ags

# ANTIGEN ANTIBODY REACTIONS PART – II

## Agglutination reactions 00:00:10

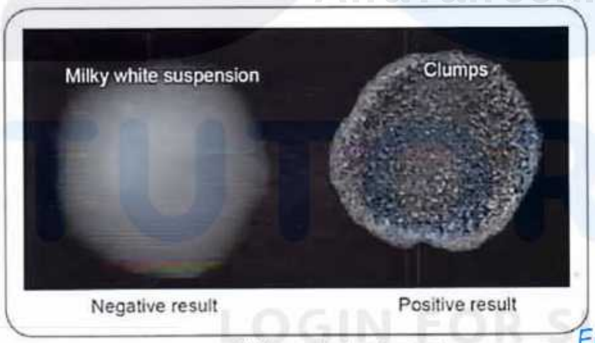
- Reaction between a particulate Ag and its corresponding Ab at optimum conditions
- More sensitive than precipitation reaction

## Direct Agglutination Reaction 00:02:10

- Ag directly interacts with antibody
- 2 types

  i. Slide agglutination reaction

  ii. Tube agglutination reaction


Milky white suspension
Clumps
Negative result
Positive result

Slide agglutination test

Fig: 1

**Example: -**

- WIDAL test
- Standard Agglutination Test or SAT (Acute Brucellosis)
- West Felix reaction
- Paul Bunnell test
- Cold agglutination test

## Microscopic Agglutination Reaction 00:04:35

- Test is done in a microtiter plate and the result is read under microscope

  Example: - Microscopic Agglutination Test (MAT) is done for diagnosis of leptospirosis.

## Indirect/Passive Agglutination test 00:06:05

- Used for Ab-detection

  Soluble Ag-is coated on a carrier molecule (RBC, latex particle, Bentonite)

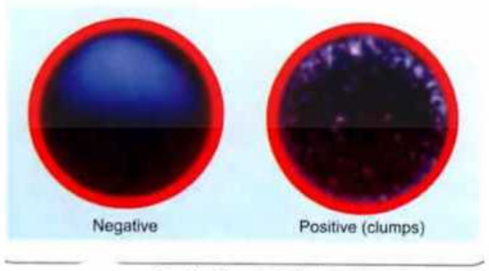
  ↓ + serum sample of patient (with Ab)

  Visible clumps are formed.

  Agglutination occurs on the surface of the molecule.

  Example: - Latex Agglutination Reaction
- Polystyrene latex particles are used (0.1microns) as carrier molecules

  - These can adsorb many antigens



Passive (latex) agglutination test

Fig: 2

- Used for the detection of Anti Streptolysin-O (ASO).

## Reverse Passive Agglutination 00:10:14

- In this Abs are attached to the carrier molecule
- Carrier molecule – Ab complex

↓ + serum of patient (with Ag)

If Ag is present in the serum, it binds to Ab on the carrier molecule

## Complement fixation test (CFT) 00:11:42

**Examples include:**

- Wasserman test: First CFT to be devised for Syphilis.
- Treponema Pallidum Immobilization (TPI) test
- Sabin Feldman dye test for toxoplasmosis

↓

Gold standard test for Toxoplasmosis.

Complement Fixation Test (CFT)

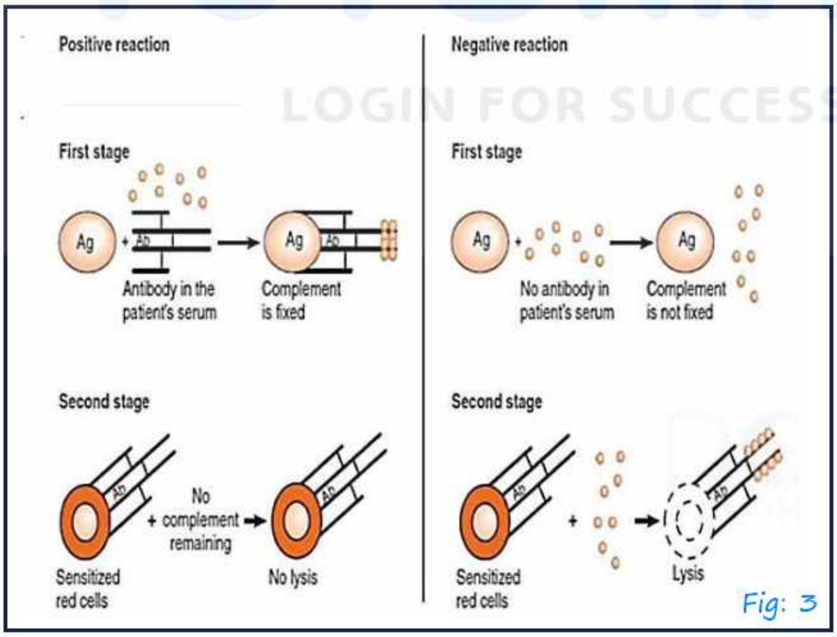

Positive reaction
First stage
Ag
Ab
Antibody in the patient's serum
Ag
Ab
Complement is fixed
Second stage
Ab
Sensitized red cells
No complement remaining
Ab
No lysis
Negative reaction
First stage
Ag
No antibody in patient's serum
Ag
Complement is not fixed
Second stage
Ab
Sensitized red cells
Ab
Lysis

Fig: 3

# ANTIGEN ANTIBODY REACTIONS PART - III

## Enzyme Linked Immune Sorbent Assay/ELISA

00:00:19

- It is used to detect Ag or Ab in serum:
- "Immune sorbent" – Ag/Ab adsorb onto surface usually made polystyrene/polyvinyl
- Elisa: also done in microtiter plates (96 wells)
- Elisa: 2 types

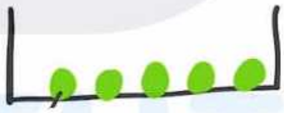
  i. Direct Elisa – for Ag-detection

  ii. Indirect Elisa – for Ab-detection

## Direct ELISA

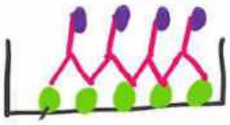
00:02:23

1)



↓ Incubation + washing

2)

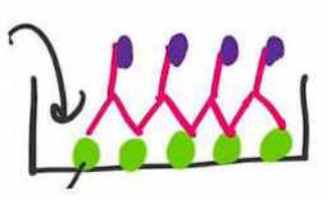


Enzyme conjugated Ab (1° Ab conjugate)

↓ Incubation + washing

substrate

3)

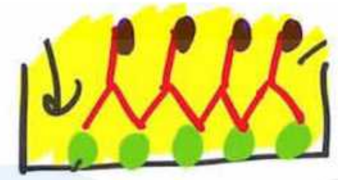


Addition of substrate

Enzyme acts on the substrate and activates it

Incubation + washing

Add chromogen



- Chromogen, needs an activated substrate

  Therefore, color changes in the well.
- Sometimes substrate itself is chromogenic in nature

| Enzymes used in ELISA and their substrate | | |
|---|---|---|
| Enzyme | Substrate | Chromogen |
| Horseradish Peroxidase | Hydrogen peroxide | Tetramethyl benzidine (TMB) |
| Urease | Urea | Bromocresol |
| B-Galactosidase | ONPG* | ONPG* |
| Alkaline Phosphatase | pNPP** | pNPP** |

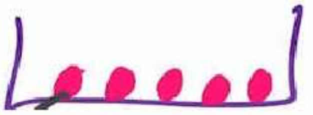
*ONPG (o-nitrophenyl-D-galactopyranoside) is cleaved by the enzyme B-galactosidase into galactose and o-nitrophenol.

**para-Nitrophenyl phosphate is a chromogenic substrate, converted to para Nitrophenol (color product).

## Indirect ELISA 00:09:10

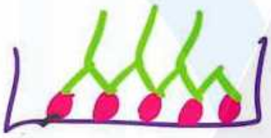
- It is done for Ab-detection

1)


Microtiter well

Ag in serum

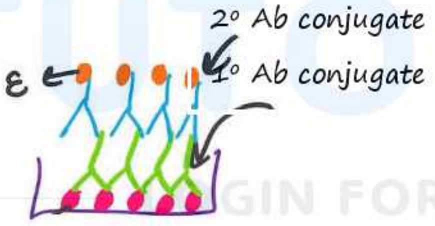
2)



Add serum (containing Ab)

Incubation + wash

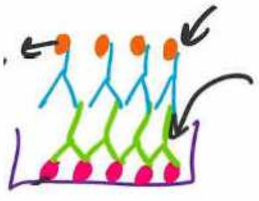
3)


2° Ab conjugate
1° Ab conjugate
E

Enzyme-linked Ab is added

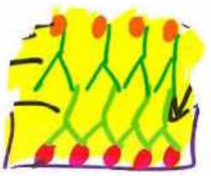
Incubation + wash

4)



Substrate-chromogen is added

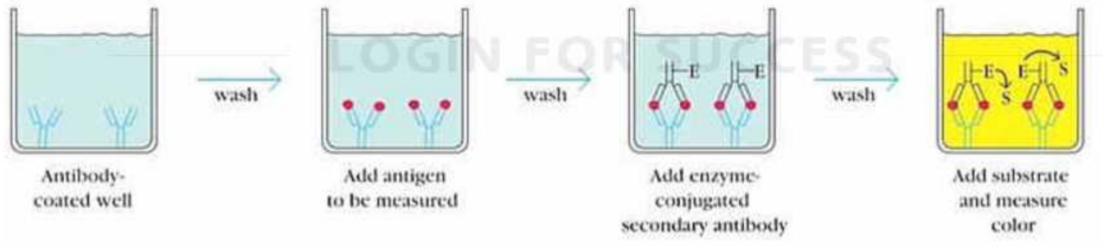
5)



Color of the well changes

- If no Abs in the serum of the patient, then when 2° Ab-conjugate is added no binding happens
- And when substrate-chromogen was added, no color change occurs.

## Sandwich ELISA 00:12:53


wash
wash
wash
E
E
E
S
E
S
Antibody-coated well
Add antigen to be measured
Add enzyme-conjugated secondary antibody
Add substrate and measure color

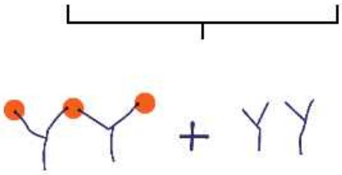
- It is a variety of direct ELISA
- Used for Ag detection in serum

## Competitive ELISA 00:14:43

- Used to detect Ag in the serum of the patient

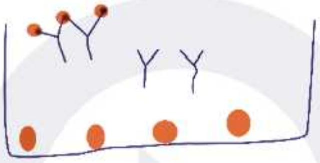
i. Serum of patient containing Ag ●●● + YYYYY corresponding Ab

ii.



Now this added to microtiter well containing Ags

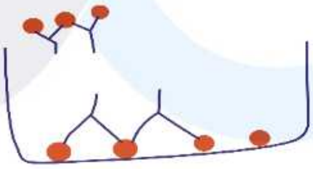
iii.



Well pre-coated with Ag

↓

iv.

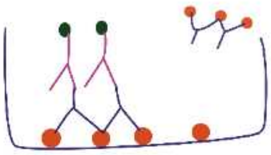


Free Ab bind to the well Ag

Enzyme linked abs are added.

↓

v.



↓ + substrate -chronogen

Increase intensity of color indicates decrease Ag in serum

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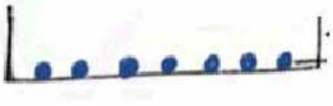
## Immunofluorescence 00:21:22

- Two types – Direct and Indirect

## Direct immunofluorescence 00:21:29

- Ag in serum sample and corresponding Ab is added

1)

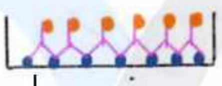


Patients' serum is added (with Ag)

Microtiter well

Ags – attached to bottom

2)



Add corresponding Ab

Tagged with a fluorochrome to the well

Incubation + wash

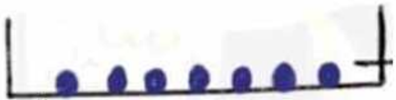
Fluorescence seen in fluorescent microscope

- If no Ag was present, then tagged Ab would have been washed away-no fluorescence was seen

## Indirect immunofluorescence (IIR) 00:22:44

- Ag in the serum, corresponding Ab is added to the serum.

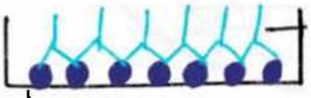
1)



Microtiter well is coated with Ags at the bottom

Add serum of the patient

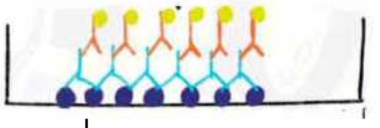
2)



Incubation + wash

Add another Ab tagged with a fluorochrome

3)



Incubation + wash

Fluorescence seen in fluorescent microscope

- If no Ab was present in the serum of the patient, then the fluorochrome tagged Ab would have been washed away. Then no fluorescence.

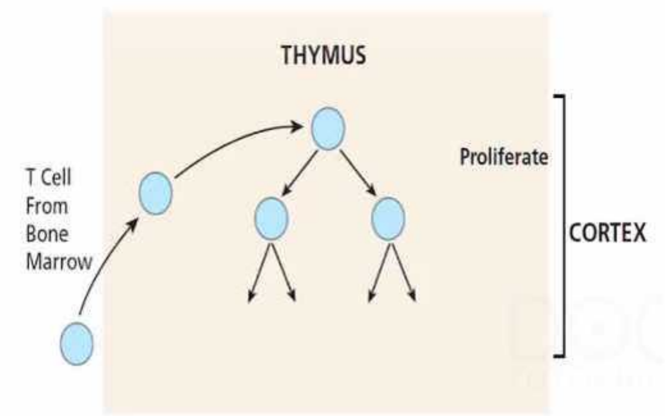
# TOLERANCE

## Tolerance 00:00:18

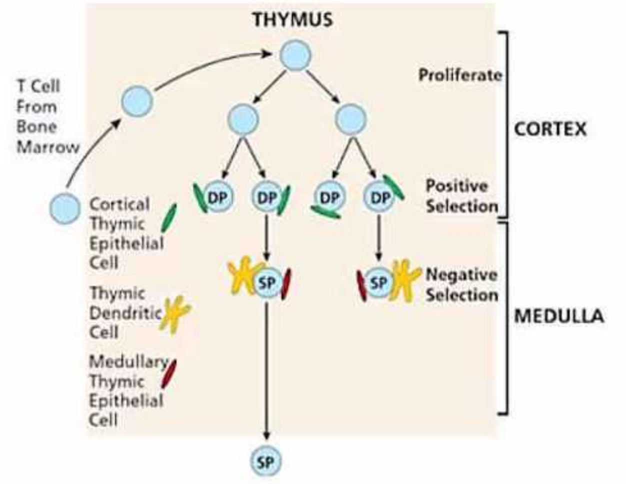
- Tolerogen – An antigen which induces immunological unresponsiveness towards it.
  - We are tolerant to our own proteins.
  - Two types
    i. Central
    ii. Peripheral

## Central Tolerance 00:03:48

- Takes place primarily in thymus.
- Thymus is responsible for generating a population of T-cells which are tolerant to self proteins.
- Bone marrow releases immature T cells → blood
  ↓
  and become mature cells ← Thymus
- Thymus – has no incoming lymphatics



Test 1 – Test of MHC restriction i.e., positive selection


THYMUS
T Cell From Bone Marrow
Proliferate
CORTEX
Cortical Thymic Epithelial Cell
DP
DP
DP
DP
Positive Selection
Thymic Dendritic Cell
SP
SP
Negative Selection
MEDULLA
Medullary Thymic Epithelial Cell
SP

Test 2 – Test of self-tolerance i.e., Negative selection

## Positive selection (survival):

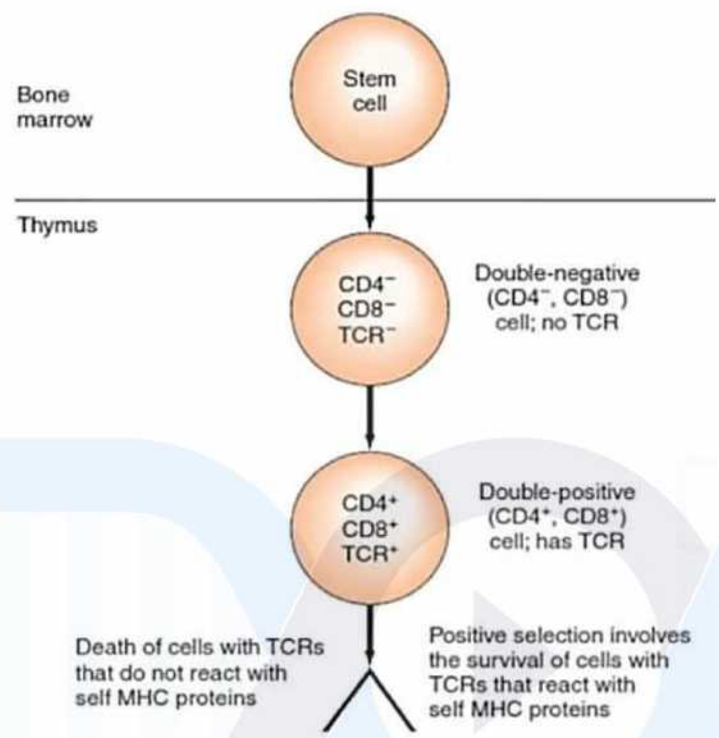
- Positive selection (survival) of T-cells results from a relatively weak interaction between TCRs and MHC self-peptide displayed on cortical thymic epithelial cells.

## Negative selection (Death):

- Is induced by a strong interaction between TCRs and MHC self-peptide expressed on bone marrow derived, thymic dendritic cells or medullary thymic epithelial cells.
  - And activation of T-cells after they leave the thymus results from a strong interaction between TCRs and MHC peptide displayed by professional antigen presenting cells.

- Thymic epithelial cells express a transcription factor known as AIRE (Autoimmune Regulator)

AIRE – Ensures the expression of self-peptides and presentation self-peptides to developing T-cells.


Bone marrow
Stem cell
Thymus
CD4⁻ CD8⁻ TCR⁻
Double-negative (CD4⁻, CD8⁻) cell; no TCR
CD4⁺ CD8⁺ TCR⁺
Double-positive (CD4⁺, CD8⁺) cell; has TCR
Death of cells with TCRs that do not react with self MHC proteins
Positive selection involves the survival of cells with TCRs that react with self MHC proteins

## Peripheral Tolerance 00:29:29

### Mechanism of peripheral tolerance

i. Clonal anergy – indifference

- It is because of absence of co-stimulation

### B-cell tolerance

i. During B-cell development, the self-reactive B-cells die in bone marrow during their development called as clonal deletion

- Clonal deletion is a way to present autoimmune disease.

ii. Receptor editing – a change occurs in the light chain of BCR so that self-reactive B-cell becomes unable to bind to any self-molecule.

# ORGANISMS NOT SEEN ON GRAM STAIN – MYCOPLASMA

## Organism which can't be seen on Gram Stain

00:00:12

- Mycoplasma
- Rickettsiae
- Chlamydiae
- Spirochaetes

## Mycoplasma

00:02:00

- **The smallest medically important bacterium**
- **Only bacterium – which has sterols in cell membrane**
- **Does not have cell wall**
- **Does not stain with Gram Stain**
- It can be stained with **Giemsa Stain**
- Knowns as **PPLO-Pleuropneumonia Like Organisms**
- Not affected by **Penicillins, Cephalosporins**

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## Mycoplasmataceae family

Has 2Genera

| Mycoplasma | Ureaplasma |
|---|---|
| ↓ | ↓ |
| Urease negative | Urease positive |
| • M.pneumoniae | • U.urealyticum |
| • M.hominis | |

- M.hominis
- U.urealyticum

] Together both constitute the 2nd most common cause of Non-Gonococcal Urethritis (NGU)

---

## M. pneumoniae 00:07:09

---

- Also known as **Eaton's agent**
- Colony: Fried egg colonies
  - Takes 2-4 weeks to appear on culture media
- **Diene's Method**: used to visualise the colonies
- Disease: **Atypical pneumonia/ Walking pneumonia**

↓

**Person** – not much symptomatic

except dry cough, mild fever

**Chest x-ray:** Lot of Infiltrates

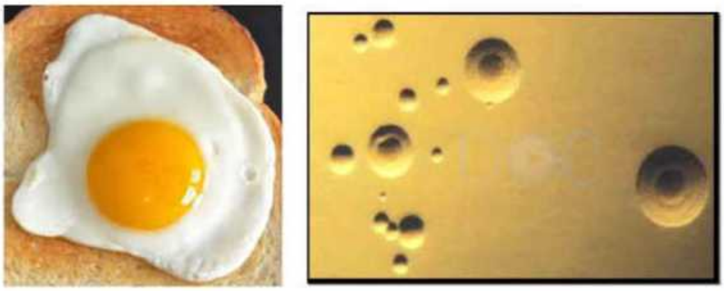

Fig: 1

Fried egg colonies of Mycoplasma pneumoniae

## Diagnosis of walking pneumonia

**Non specific Ab-detection tests:**

- For tentative diagnosis
- Also known as **Heterophile agglutination tests**

**Specific Ab-detection tests:**

- Are Confirmatory tests

### Non-specific Ab test:

- Cold agglutinin test: Detects antibodies which cross-react with human blood group 'O' RBCs at 4°C
- Streptococcus MG Test: Detects antibodies which cross-react with group F streptococcus

### Specific Ab detection can be done by:

- ELISA
- Indirect Immunofluorescence
- Haemagglutination test

### Treatment

- Macrolides
- Quinolones

# ORGANISMS NOT SEEN ON GRAM STAIN-RICKETTSIAE

## Rickettsiae 00:00:35

- Atypical bacteria – obligate intracellular organisms
- Therefore, can't grow on artificial culture media
- Infect vascular endothelial cells; produce skin rash
- Rash is absent in Q-fever
- Most species are transmitted by – Arthropod vector
- Animal reservoirs present
- Zoonotic diseases

## Disease-Groups 00:05:30

I.

**Typhus-fever group**

| **Epidemic Typhus** | **Endemic Typhus** |
|---|---|
| • Caused by R.prowazekii | • Caused by R.typhi/ R.mooseri |
| • Vector-Human Body cause | • Vector: Rat flea |
| • Not a zoonotic disease | • Animal reservoir: rodents |
| • Infects humans only | • Less severe than epidemic typhus |
| • Also known as 'Jail fever' | |
| • Re-activation typhus<br>↓<br>**Recrudescent typhus /Brill Zinsser's disease**<br>- Less severe than epidemic typhus<br>- No vector | |

## Clinical features of Rickettsial diseases:

- Fever
- Prostration
- Rash
- Myalgia

## II. Spotted fever group

| Disease | Vector | Causative bacteria |
|---|---|---|
| 1. RMSF<br>Rockey Mountain Spotted fever<br>•Extremely/most severe rickettsial disease | Hard tick | R. rickettsii |

- In RMSF, rash first appears on extremities, then appears on trunk in contrast to other Rickettsial diseases.

| Disease | Vector | Causative bacteria |
|---|---|---|
| 2.Indian tick typhus | Hard tick | R.conori |
| 3.Australian tick typhus | Hard tick | R. australis |
| 4.Siberian tick typhus | Hard tick | R.siberica |
| 5. Rickettsial pox | Mite | R.akari |

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- Mildest rickettsial disease overall: Rickettsial pox

### iii. Scrub typhus

- Fever
- Rash
- Lymphadenopathy
    - Caused by Orientia tsutsugamushi
    - Vector: **Larvae of Trombiculid mite**

↓

"Chiggers"

- Disease also known as – Chiggerosis

### Zoonotic tetrad:

- Vector
- Reservoir: Field mice, Rodents
- Scrubby vegetation
- Wet season (mite lays eggs)

### III. Q-fever

- No rash
- Non-arthropod borne
- Animal reservoir-present
- Zoonotic disease

- Infection occurs by – 3Is
  - **Inoculation (Vets, Butchers)**
  - **Inhalation of dried-up secretions, excreta of infected animals**
  - **Ingestion of meat of Infected animals**

- Caused by Coxiella burnetti
- Clinical features:
  - Fever alone
  - Pneumonia
  - Infective endocarditis
  - Hepatitis
- Recently, It has been made possible to grow it in cell free media.

## Diagnosis:

### Antibody detection

**Specific Ab**

1. ELISA
2. Indirect Immuno-fluorescence

**Non-specific Ab**

- Detection tests/: heterophile agglutination tests
- Eg: Weil felix reaction

-Tube agglutination test

-Principle: Sharing of an alkali-stable carbohydrate antigen between certain Rickettsial species and non-motile strains of Proteus

Non motile strains of proteus

-Eg: OX-K, OX-19, OX-2

-X → Absence of H-Ag

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- Between NON-MOTILE strains of Proteus and some rickettsiae – "Carbohydrate Ag" is common

## Treatment:

- Tetracycline/ Chloramphenicol.

# ORGANISMS NOT SEEN ON GRAM STAIN – CHLAMYDIAE

## Chalmydiae 00:00:24

- Atypical bacteria
- Can't grow on Artificial Media, since they are obligate intracellular
- Also known as ATP parasites/ energy parasites
- Also known as Basophilic viruses
  - Like viruses, they pass through membrane filters
  - Basophilic Inclusion Bodies
- Cell wall-made up of proteins
- Exists in 2-morphological forms

| **Elementary body** | **Reticulate body** |
|---|---|
| Extracellular infective form | Intracellular replicative form (By Binary fission) |
| Smaller | Bigger |
| Less RNA | More RNA |

### Life cycle of Chlamydiae

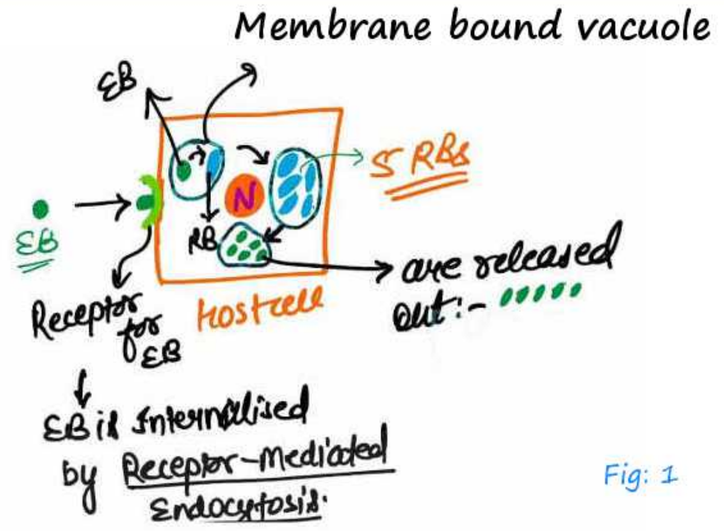
Membrane bound vacuole
EB
EB
5 RBs
N
RB
are released out:-
Receptor for EB
Host cell
↓
EB is Internalised by Receptor-Mediated Endocytosis.

Fig: 1

- Inclusion bodies: Membrane bound vacuoles containing EB or EB+RB or RB in the infected host cells

↓

- Detected under microscope

## 3 important species 00:12:47

### Chlamydia trachomatis:

- Divided into 15 serovars/ serotypes
- Clubbed into 3-Groups

| A, B, $B_a$, C | D to K | L1, L2, L3 |
|---|---|---|
| •Cause- trachoma<br>↓<br>Chronic conjunctivitis | •Most common cause of Non-Gonococcal Urethritis (NGU) | |

| | | |
|---|---|---|
| • In India: It is the most common infectious cause of blindness | • Inclusion conjunctivitis (does not cause blindness)<br>• Ophthalmia neonatorum<br>• Infant pneumonia | |

### D-K

i. NGU-STI
  - Males:
    - Symptomatic
    - Urethral discharge
  - Females:
    - Asymptomatic
    - Endocervicitis

ii. Inclusion conjunctivitis

iii. Ophthalmia neonatorum

iv. Infant pneumonia

### $L_1$, $L_2$, $L_3$

- Cause Sexually Transmitted Infection (STI)
- Lymphogranuloma Venereum (LGV) → Genital Ulcer
- $L_2$ – most common cause
- Genital ulcer can be painful/ painless
- Genital Ulcer is associated with painful inguinal lymphadenopathy

## Diagnosis of C.trachomatis infections :

i. Microscopy to look for Inclusion Bodies (IBs) – less sensitive

- Giemsa stain – stains IBs of all – 3 species:
  - C.trachomatis
  - Chlamydophila pneumoniae
  - Chlamydophila psittaci
- Iodine stain: stains IBs of – C.trachomatis

  (Because IBs of C.trachomatis have glycogen )
- Direct fluorescent antibody (DFA) stain
  - Best stain to see IBs
  - Highly specific

ii. Culture:

- In the Yolk sac of fertilised/ embryonated hen's egg
  - Hela cell line
  - Hep-2 cell line
  - $M_C$Coy cell line

iii. NAAT (Nucleic Acid Amplification Test):

- Most sensitive & specific hence gold standard
- PCR is used commonly

---

## Chlamydophila psittaci 00:25:03

- Psittacosis – Infection of birds of psittacine family (parrots)
- Ornithosis – Infection of birds

- In humans – pneumonia → no man-to-man transmission
- This pneumonia: Zoonotic disease
  - Transmitted from birds to man, not man to man
- Infection is acquired by Inhalation of dried-up excreta/ secretions of birds which become aerosolised
- NOTE: Pneumonic plague is the only zoonotic disease which can be transmitted from man to man.

## Diagnosis:

- Complement Fixation Test (CFT)
- Micro Immunofluorescence Test (MIF)

## Chlamydophila pneumoniae:

- Only 1 serotype
- Cause pneumonia in humans
- Not a zoonotic disease
- Transmission from man to man + nt
- Toughest of all 3 species to culture
- Thought to play a role in:
  - Atherosclerosis
  - Asthma
  - Sarcoidosis
- 3rd most common cause of Community Acquired Pneumonia (CAP) in humans

## Causes of CAP in humans:

- Overall, most common cause – Pneumococcus/ Streptococcus pneumoniae
- 2nd most common cause – Haemophilus influenzae (non-capsulated strains)
- 3rd most common cause – Chlamydophila pneumoniae

## Treatment of chlamydial infections:

- Macrolides
- Fluoroquinolones.

# ORGANISM NOT SEEN ON GRAM STAIN – SPIROCHAETES

## Spirochaetes 00:00:24

- Spiral organisms
- Genera:
  - Borrelia
  - Leptospira
  - Treponema
- Gram negative cell structure

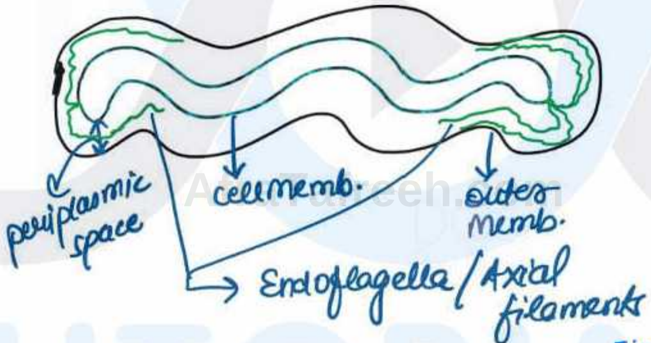
periplasmic space
cell memb.
outer memb.
Endoflagella/Axial filaments

Fig: 1

- Motile organisms
- Types of motility:
  - Corkscrew type
  - Flexion – Extension type
  - Translatory motion/ spring like action

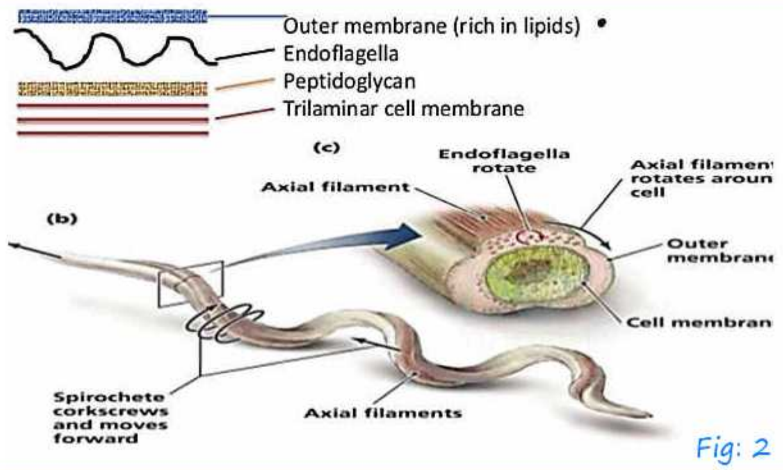
Outer membrane (rich in lipids)
Endoflagella
Peptidoglycan
Trilaminar cell membrane
(c)
Endoflagella rotate
Axial filament
Axial filament rotates aroun cell
(b)
Outer membran
Cell membran
Spirochete corkscrews and moves forward
Axial filaments

Fig: 2

- Spirochaetes are seen on Dark Ground microscopy

## Borrelia: 00:07:16

- Big enough to be seen under microscope
- Stained with Giemsa stain
- Has loose spirals

Diseases

Lyme disease

Relapsing fever

## Lyme disease: 00:08:28

- By Borrelia burgdorferi
- Vector: Hard tick
- Most common vector borne disease in the West
- I.P: 3-32days

### Clinical features:

i. Early localized stage:

- Single skin lesion known as **Erythema Chronicum Migrans (ECM)**

  (or)

  **Bull's Eye Rash/ Target Rash**
- This skin lesion is seen at site of Tick Bite

ii. **Early disseminated stage:**

a. Characterized by multiple skin lesions (satellite lesions)
  - Smaller than ECM

b. CVS – fluctuating degree of AV Block
  - Myocarditis

c. CNS – Neuropathies
  - Sometimes meningitis
  - Facial nerve palsy

d. Joints – Oligoarticular arthritis
  - Most common joint affected – Knee joint

iii. **Late stage:** Neuropsychiatric manifestations

## Diagnosis:

- Tough to culture
  - Medium: BSK-II
- Ab-detection by ELISA – Is the mainstay
  - IgM detection or 4-fold or more increase in IgG detection

**Treatment:**

- Ceftriaxone

## Relapsing fever: 00:15:46

**Relapsing fever**

| Epidemic relapsing fever | Endemic relapsing fever |
|---|---|
| • Vector: Louse | • Vector: Soft tick |
| • Caused by:<br>B.recurrentis recurrentis | • Caused by:<br>– B. duttoni<br>– B. turicatae<br>– B. hermsii |

## Leptospira 00:17:26

- Tightest spirals
- Hooked ends – facing in opposite direction
- Disease: Leptospirosis

**Leptospirosis:**

- Most common zoonotic disease on earth
- Also known as "Rat fever"/ "Paddy fever"/ "100-day fever"
- Severe form of leptospirosis is called as **Weil's disease or Icterohaemorrhagic fever**
- It is associated with 3 R's
  - Rodents – most important reservoir

  - Rice fields/ Paddy fields
  - Rainfall
- Bacterium multiples asymptomatically in renal tubules of rodents

↓

Shed in the rodent urine

- During heavy rains/ floods

↓

Rodents contaminate surface water with their urine

- Humans get infected via contact of mucous membrane or broken/ breached skin with:
  - Contaminated $H_2O$
  - Infected tissues of rodents

**But not following Rodent Bite**

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- Clinical features:
  - i. Asymptomatic
  - ii. Fever only
  - iii. Sometimes **Weil's disease/ Icterohaemorrhagic fever**

**Characterized by Hepatorenal syndrome**

- Pathology of leptospirosis is vasculitis
- Important pathological features in kidney:
  - Interstitial nephritis
  - Tubular necrosis

- Important pathological feature in liver: Centrilobular necrosis

## Diagnosis:

i. Microscopy of:
  - Blood: Organisms are present in the blood during 1st week of disease
  - Urine: Organisms are shed for longer periods
  - Less sensitive

ii. Culture on:
  - Fletcher's medium
  - EMJH medium
  - Korthof's medium

iii. Serology: is gold standard method
  - Microscopic Agglutination Test (MAT): Detects Ab in the serum

## Treatment:

- Penicillins
- Tetracyclines

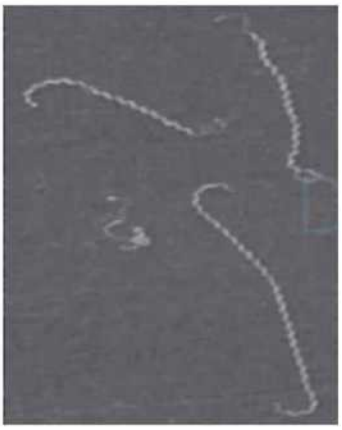

Fig: 3

Transmission electron micrograph of L. interrogans

## Treponema 00:29:29

- Treponema – ("Trepo"-to turn, "nema"-Thread)

**Treponema**

| **Pathogenic treponemes:** | **Non-Pathogenic treponemes:** |
|---|---|
| • Not normally present in humans | • Commensals in oral cavity, genitourinary tract |
| • Cause disease | • No disease |
| **Examples:** | |
| 1. Treponema pallidum subspecies pallidum | T. phagedenis – Reiter's strain |
| – Causes venereal syphilis | |
| 2. T. pallidum subspecies endemicum | |
| – Causes endemic syphilis | |

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3. T. pallidum subspecies pertenue
   - Cause yaws
4. T. pallidum subspecies carateum
   - Causes pinta

- Treponema pallidum subspecies pallidum → referred to as "T. pallidum"

## Treponema pallidum 00:29:29

- Disease – syphilis
- Most common mode of infection:

  1. Sexual intercourse → Venereal syphilis

- Other modes:

  2. Mother to child via placenta (Transplacental) → Congenital syphilis
  3. Blood transfusion

> - Blood in blood banks is to be screened for the following infections:
> - Treponema pallidum
> - Plasmodium species – malaria
> - HIV
> - HBV, HCV
> - HCV

4. Organ transplantation

## Venereal syphilis 00:36:58

- It is a sexually transmitted infection
- I.P: 9–90 days

Stages:

i. Primary syphilis:

- Painless, hard/ Indurated genital ulcer known as Hard chancre/ Hunterian chancre
- "Sir John Hunter" – distinguished Gonorrhoea from syphilis
- Painless inguinal lymphadenopathy
- Highly infectious lesions

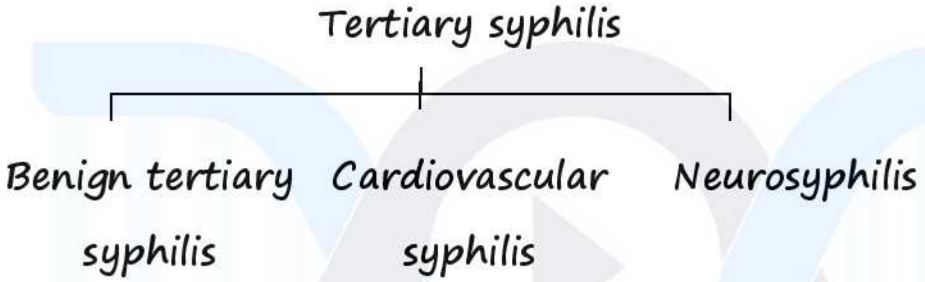
ii. Secondary syphilis:

- Bacteria are present in the blood
- It is a systemic infection
- Features:
  - Generalised skin rash (Great POX)
  - Generalised lymphadenopathy
  - Oral mucous patches
  - Multiple genital painless lesions known as "Condyloma lata"
- If not treated, lesions resolve, but it does not mean cure and patient enters the latent stage
- Latent syphilis stage:

- There are no clinical features, but positive serological tests
- Symptoms can relapse

Referred as "Waxing & Waning course" of the disease

iii. Tertiary syphilis:


Tertiary syphilis
Benign tertiary syphilis
Cardiovascular syphilis
Neurosyphilis

a. Benign tertiary syphilis:

- Lesions are known as "Gummas"
- Gummas are Granulomatous lesions
- Most common sites:
  - Bones – deep gnawing pain
  - Skin – painless lesions
- Gummas are:
  - Non-infectious
  - Resolve with therapy

b. Cardiovascular syphilis:

- Pathology is obliterative endarteritis of vasa vasorum supplying the:
  - Aorta (ascending)

- Arch of aorta

Resulting in aortic aneurysm → "Syphilitic aneurysm"

Aortic regurgitation – If aortic valve ring is involved

c. Neurosyphilis:

- Presentation can be

1. Aseptic meningitis

**Also caused by Mycobacterium tuberculosis**

2. Stroke – if blood vessels of brain are involved
3. Tabes dorsalis – if spinal cord (posterior column + dorsal roots) are involved
4. Neuropsychiatric illness: General paresis (of the Insane)

Diagnosis:

1. Microscopy: For primary and secondary stages

a. Dark ground microscopy: To see the motility

- Disadvantages:
  - Not available everywhere
  - Smear should be examined immediately else organisms will die
  - Cannot differentiate between pathogenic and non-pathogenic treponemes

b. Direct Fluorescent Antibody (DFA) stain:

- Highly specific (Based on monoclonal Abs)

- Need not examine the smear immediately

c. Giemsa stain:

- Pale colour on Giemsa stain

2. Serology:

- For secondary & tertiary stages
- Ab detection

Serology

| **Nonspecific/ Non-treponemal tests:** | **Specific/ Treponemal tests** |
|---|---|
| Ag used: Cardiolipin<br>Ag from Ox/ beef heart | Ag used: Specific for T. pallidum<br>• Ag used is obtained from pathogenic strain – Nichol's strain<br>• Nichol's strain – can be maintained by serial inoculation in rabbit's testis – Intratesticular passage |

- Nonpathogenic treponemes **can grow** on artificial media
- Pathogenic treponemes can't **grow on** artificial media

### Non-specific/ Non-treponemal tests:

1. Wasserman test: It is CFT (not used now)
2. Kahn's test: It is a tube flocculation/ tube precipitation test
3. VDRL test: Is a slide flocculation/ slide precipitation test

- VDRL = Venereal Disease Research Laboratory

4. RPR test – Rapid Plasma Reagin test:
   - Modification of VDRL test
     - VDRL – can be done on CSF sample. Therefore, neurosyphilis can be diagnosed
     - RPR – Can't be done on CSF sample

### Samples for VDRL & RPR:

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| VDRL | RPR |
|---|---|
| • Can be done on CSF sample. Therefore, neurosyphilis can be diagnosed<br>• Microscope is needed to comment on reactivity of test<br>• Serum should be heated at 56°c for 30 minutes | • Can't be done on CSF sample<br>• It is a modification of VDRL test<br>-Ag is coated with carbon particles. Therefore, reaction can be observed with naked eye<br>-Heating is not required |

## Advantages of Non-specific tests:

1. Cheap
2. Become negative with successful treatment. Therefore, can be used as "Follow up tests" or prognostic tests

## Disadvantages of Non-specific tests:

- Not confirmatory
- Biologically false positive reactions can occur:
  - Examples:
    1. Pregnancy
    2. Infectious mononucleosis – by EBV
    3. Viral – vaccinations
       - Viral – pneumonias
       - Viral – hepatitis
    4. Tropical diseases
       - Malaria
       - Kala azar
       - Leprosy
    5. Autoimmune diseases
       - SLE
       - R.A
    6. HIV
    7. Old age
    8. I.V drug abuse

## Specific treponemal tests:

1. Treponema Pallidum Immobilization (TPI) test
2. T. pallidum haemagglutination (TPHA) test
3. T. pallidum particle agglutination test: (TPPA)
4. FTA (Fluorescent Treponemal antibody) – Absorption test:
    - Based on principle of indirect immunofluorescence

- **Most sensitive, most specific: TPPA**

- Primary syphilis:

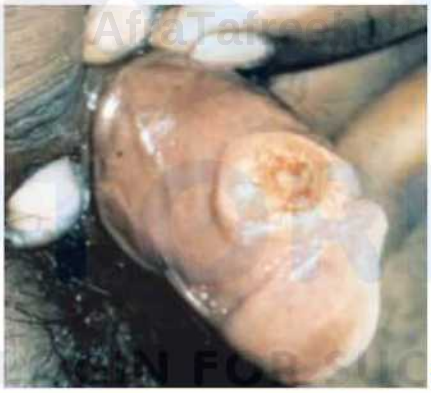

Fig: 4

- Secondary syphilis:

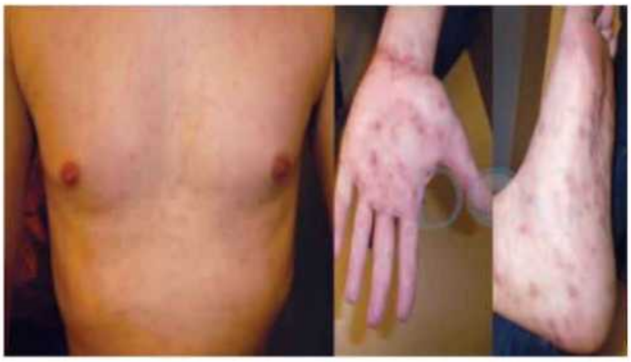

Fig: 5

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- Palmar lesions of secondary syphilis:

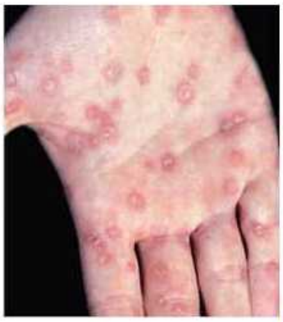

Fig: 6

- Condylomata lata of secondary syphilis:

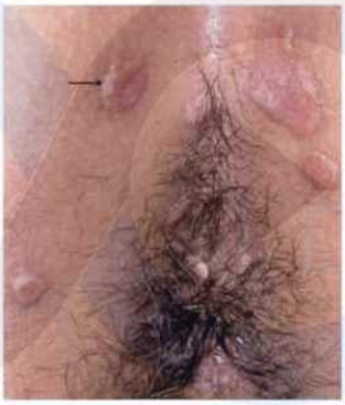

Fig: 7

- Direct microscopy of T. pallidum:

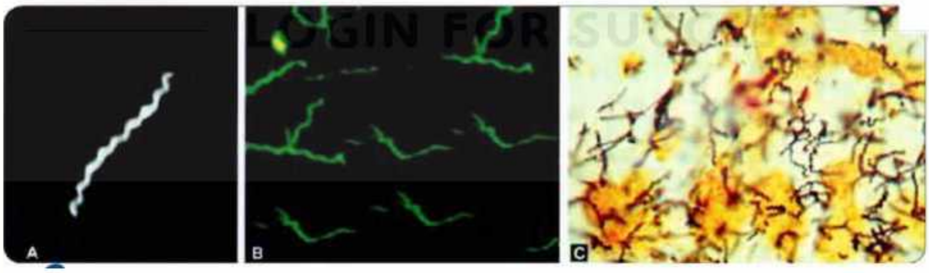

Fig: 8

- Direct microscopy T. pallidum A. Dark ground microscope; B. Direct fluorescent antibody staining (DFA-TP) and C. silver impregnation method

- Erythema chronicum migrans-rash of lyme disease:

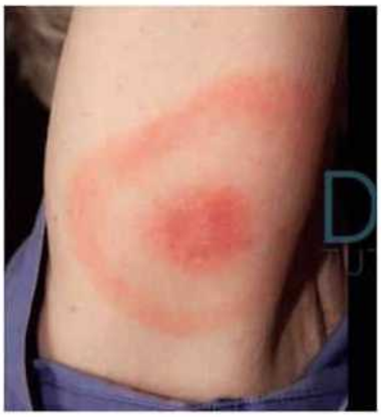

Fig: 9

- VDRL slide:

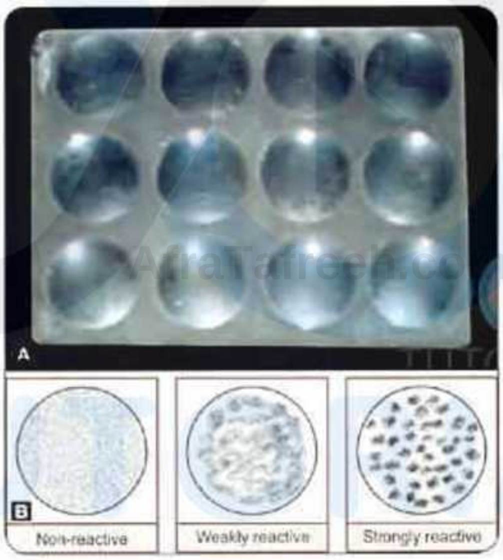
A
B
Non-reactive
Weakly reactive
Strongly reactive

Fig: 10

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- Giemsa stain – Borrelia:

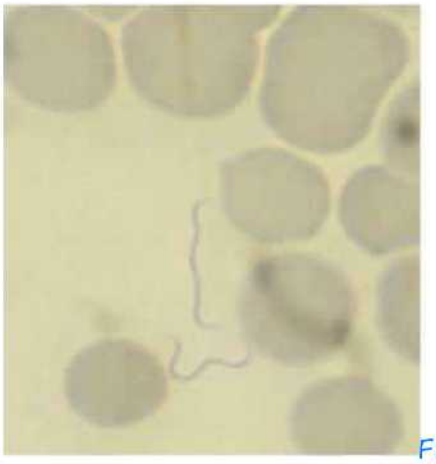

Fig: 11

## Advantages of treponemal tests:

- Confirmatory tests

## Disadvantages of specific tests:

1. Expensive
2. Remain positive for weeks even after successful treatment – can't be used for follow up

| RPR | TPPA | Result |
|---|---|---|
| + | + | Syphilis |
| + | – | Biologically false positive |
| – | + | Cure in most patients, but it can also be "not cured", since 30% of patients of syphilis who do not get any treatment become VDRL negative |

## Treatment:

- DOC: Penicillin
  - If penicillin allergy: Macrolides
  - In penicillins: Benzathine penicillin (long acting) is preferred because generation time of T. pallidum is 30 hours.

# ENTEROBACTERIACEAE FAMILY-SALMONELLA

## Salmonella 00:00:13

- Pathogen, never commensal
- Usually, Motile
- Gram negative rods
- NLF
- Ferments glucose → Acid + Gas (Aerogenic)
  - Except S.typhi → which is anaerogenic
- I M Vi C

  − + − +

  Citrate Test (C) – is negative in S.typhi and S.paratyphi A

- They are $H_2S$ producers – Except S.paratyphi A
- Non motile species:
  - S.gallinarum
  - S.pullorum

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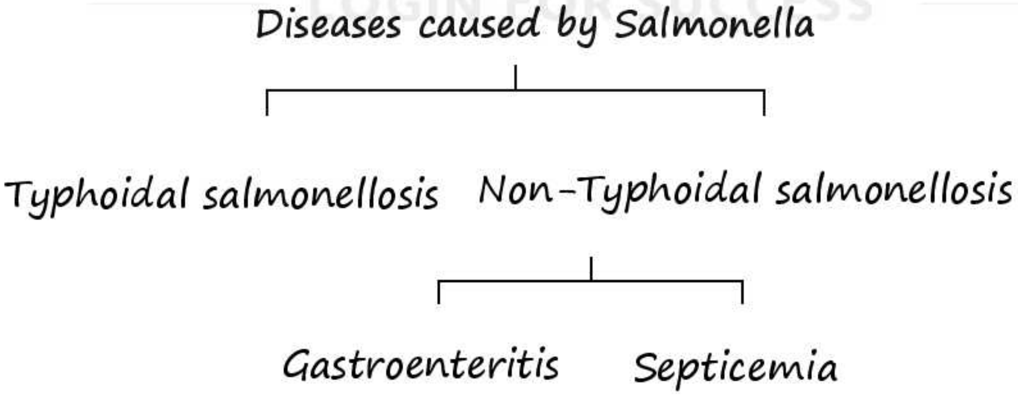
## Diseases caused by Salmonella 00:04:54


Diseases caused by Salmonella
Typhoidal salmonellosis
Non-Typhoidal salmonellosis
Gastroenteritis
Septicemia

### Typhoidal salmonellosis: 'Enteric Fever'

- MCC – S. typhi
- 2nd MCC – S. paratyphi A

- 3rd MCC – S. paratyphi B
- Rarest – S. paratyphi C → not seen in India

- All these are human pathogens only with no animal reservoir
- Source of Infection: Cases or carriers

## Non-Typhoidal salmonellosis:

Non-typhoidal salmonellosis

Gastroenteritis Septicaemia

i. Gastroenteritis:
  - I.P > 16-18 hours
  - Causes:
    - S. typhimurium
    - S. enteritidis
  - Foods:
    - Meat & meat products
    - Poultry
    - Milk and milk products

ii. Septicaemia:
  - By S. cholerasuis
  - Reservior – pigs

## Enteric fever 00:10:40

- I.P: 1-2 weeks
- Mode of Infection – Feco-oral route
- Following ingestion: Bacteria are taken up by Macrophages

In the gut
↓
Bacteria resist killing
↓
Enter portal blood (primary bacteremia)
↓
Bacteria travel to RES which are rich in macrophages
↓
Invade-liver, spleen, bone marrow;
Multiply inside the macrophages
↓
Enter blood (secondary bacteremia)
↓
Now symptoms & signs develop in the patient

- IP → 1-2 weeks

**Clinical features:**

- Stepladder pyrexia
- Rose spots
- Headache, chills cough
- Myalgia, arthralgia
- Abdominal pain, nausea, vomiting, anorexia
- Hepatosplenomegaly
- Complications:
  - GI bleeding, Intestinal perforation
  - Rarely neurologic features
- Relative bradycardia
  - Due to inhibitory effect on SA Node
- Complications after 3rd week of fever
- Some organisms seed gall bladder-passed out in faeces
- Some organisms are shed in urine

## Diagnosis of enteric fever 00:16:17

### I. Isolation/ culture of organism:

- Samples:
  - Blood
  - Faeces
  - Urine
- Blood culture:
  - Gold standard
  - 100% specific
  - Sensitivity varies

| | Sensitivity of blood culture |
|---|---|
| 1st week of fever | 90% |
| 2nd week of fever | 75% |
| 3rd week of fever | 60% |
| 4th week of fever | 30-40% |

- Faeces culture: Enrichment broth/ media:

  i. Selenite-F broth

  ii. Tetrathionate broth

  iii. Gram-negative broth

  ↓ incubate for 6-8 hours

### Subculture on selective media:

a. MacConkey agar: NLF/ colourless colonies

b. DCA/ Deoxycholate Citrate Agar-colourless colonies with black center

c. XLD/ Xylose Lysine Deoxycholate agar – colourless colonies with black center

d. Wilson & blair media: colourless colonies with a metallic sheen

- Urine culture: CLED & BA+MA

### II. Antigen detection in serum:

- Best done in 1st week of fever
- Because of maximum antigenemia

### III. Antibody detection in serum:

- Widal test: Tube agglutination
- Detects Abs to 'O' and 'H' Ags of Salmonella

| 'O' Ag | 'H' Ag |
|---|---|
| Somatic Ag | Flagellar Ag |
| Heat stable | Heat labile |
| Polysaccharide (PS) | Protein |
| Low Ab-titre | High Ab-titre |
| Less specific | More specific |
| Only IgM | IgM & IgG both responses are present |
| Abs come early & leave early | Abs come late, persist longer, disappear late |
| **Felix tube:** Round bottom | **Dreyer's tube:** Conical bottom |
| Agglutination – coarse granular | Cottony, fluffy agglutination |

- Abs against:
  - 'O' Ag of S. typhi
  - 'H' Ag of S. typhi
  - 'H' Ag of S. paratyphi A
  - 'H' Ag of S. paratyphi B
- Serum sample is added in all tubes
- Serum is diluted using **normal saline**

Titre: $\frac{1}{\text{maximum dilution at which Ag-Ab reaction/ agglutination occurs}}$

- Visible agglutination occurs at some dilutions, titre is taken as reciprocal of maximum dilution
- Widal test – Drawbacks
  - i. False positive tests:
    - Vaccination
    - Anamnestic reaction → Becomes negative in a week
  - ii. False negative tests:
    - Early antibiotic therapy

**Endemic titre:**

| | Titre |
|---|---|
| For 'O' Ag: | ≤ 100 |
| For 'H' Ag: | ≤ 200 |

- Widal test-interpretation: widal test should be done twice

- A-4-fold or more increase in Ab-titre between acute and convalescent sera (paired sera) → **Positive test**
- Example:

| Widal test-1 | After → 15 days | Widal test- 2 |
|---|---|---|
| O: 90 | | O:360 |
| H: 180 | | H: 720 |

- Inter-observer variation in interpretation of results – is common

### Carriers in enteric fever:

- Carrier state develops in 5% cases

**Carriers**

Convalescent  Temporary  Chronic

- Convalescent carries: Shed Bacteria for < 3months
- Temporary carries: Shed Bacteria for 3months – 1year
- Chronic carries: Shed Bacteria > 1year
  - Eg: Mary Mallon/ typhoid Mary

- Sometimes in fecal carriers – Gall bladder is removed

### How NOT to do diagnosis of carriers:

- Blood culture: If positive, it is a case
- Widal test: Negative in carriers

## How to make a diagnosis of carrier state:

- Faeces culture
- Urine culture
    - Since, there is intermittent shedding, multiple samples are collected
    - Correlated clinically
- Sewer swab technique
- Only serological test for diagnosis of carriers: Anti Vi Ab test (Vi-Virulence)

↓

Against polysaccharide Ag

## Prevention:

- Proper sewage disposal
- Proper personal hygiene

## Treatment

| | Drugs of choice | Alternate drug |
|---|---|---|
| **Empirical treatment** | This is the treatment given before antimicrobial susceptibility report is available | |
| | Ceftriaxone[a] | Azithromycin[b] |
| **Fully susceptible** | Susceptible to all the drugs given for enteric fever | |
| | Ciprofloxacin | Amoxicillin<br>Chloramphenicol<br>Cotrimoxazole |
| **MDR strains (multi-drug resistance)** | Defined as resistant to chloramphenicol, ampicillin and cotrimoxazole-antibiotics used to treat enteric fever long back | |
| | Ciprofloxacin | Ceftriaxone<br>Azithromycin |
| | Defined as strains resistant to nalidixic acid with reduced susceptibility to ciprofloxacin | |

| | | |
|---|---|---|
| **NAR strains (Nalidixic acid resistant)** | Ceftriaxone | Azithromycin, ciprofloxacin[d] (higher dose and longer course) |
| **Carriers** | Ampicillin or Amoxicillin plus probenecid for 6 weeks | Cotrimoxazole or ciprofloxacin |

a. 1-2g/day (IV) for 10-14 days
b. 1g/day oral for 5days
c. 500mg twice a day oral for 5days
d. 750mg twice a day per oral for 10-14days

# ENTEROBACTERIACEAE-SHIGELLA

## Shigella 00:00:05

- Non motile
- NLF → Except S. sonnei – Late Lactose Fermenter (LLF)
- Doesn't produce $H_2S$
- Ferments Glucose producing only acid, no gas ( Anaerogenic )
- Disease: **Bacillary dysentery**
- Species: 4 species based on mannitol fermentation
  - S. dysenteriae – Doesn't ferment mannitol
  - S. flexneri
  - S. boydii – ferment mannitol
  - S. sonnei
- Serotype-1 of S. dysenteriae: "**Shiga's bacillus**,

  Is catalase-negative

## Bacillary dysentery 00:04:43

- Mode of infection: Feco-oral route
- Infective dose = 100 organisms (Because Shigella is acid-stable)

**In case of Salmonella: Infective dose is high ≈ $10^5$-$10^8$ organisms**

**(Because Acid labile)**

- Shigella enters the gut cells NOT from the luminal side but from basolateral side, with the help of specialized cells known as "M" cells

## Diagnosis:

- Sample: Faeces
- Enrichment broth:
  - Selenite F-Broth
  - Gram-negative Broth

**Tetrathionate broth is not used for Shigella because it is toxic to it shigella**

- Incubate the Broth for **6-8 hours**
- Then subculture on **selective media:**
  - i. **MA:** NLF colonies (except S.sonnei) which is late lactose fermenter
  - ii. **DCA:** Colourless colonies
  - iii. **XLD:** Colourless colonies
- Wilson and Blair medium – Not used for Shigella

## Treatment:

- **DOC:** Ciprofloxacin

## NOTE:

- **Shigella:** Invasion of gut cells is the most important requisite for disease than toxin production
- **S. dysenteriae:** Produces Shiga toxin
  - **MOA of Shiga toxin:** Damage to 60S Ribosomes. Therefore, No protein synthesis
- **Shigella:** Locally invasive, not systemically invasive generally
- **Systemic invasion:** Seen in case of immunocompromised people
  - Eg: HIV infected people

- In India: Most common species: S. flexneri
- In the West: Most common species: S. sonnei – 1 serotype only
- Mild disease – caused by S. sonnei
  - May cause only diarrhoea, not dysentery
- Shigella: pathogen, not commensal.

# ENTEROBACTERIACEAE FAMILY-KLEBSIELLA

## Klebsiella 00:00:03

### Klebsiella

- Gram-negative, Non-motile rods (shown in figure)
- I M Vi C

  – – + +
- LF – Lactose fermenter
- Capsulated – Highly hygroscopic capsule
- On MacConkey agar: **Pink mucoid colonies** (as shown in figure)

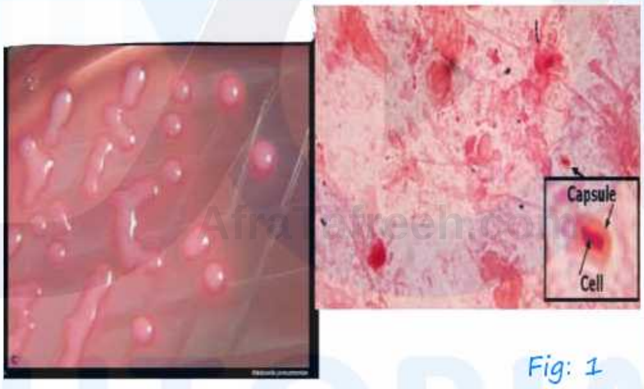


Fig: 1

- Ferments glucose → Acid + Abundant Gas
- Urease positive

### Diseases:

i. Pneumonia: In chronic bronchopulmonary disease patients and alcoholics
   - Sputum – characterized by red currant jelly sputum:
     - Red due to haemoptysis
     - Currant jelly: due to capsule

ii. UTI: usually in the setting of urinary catheterisation

iii. Septicaemia in geriatric population

iv. MCC of neonatal septicaemia and neonatal meningitis in India

### K. ozaenae:

- Causes ozaena/ atrophic rhinitis
- Atrophic rhinitis – characterized by foul-smelling nasal discharge

### K. rhinoscleromatis:

- 'Hard' swelling of 'Nose'
- Disease – rhinoscleroma/ woody nose

### K. granulomatis:

- Old name: Calymmatobacterium granulomatis
- Causes STI – **Granuloma Inguinale/ Donovonosis** characterised by
  Painless genital ulcer without inguinal lymphadenopathy
- From ulcer, smear is made + stained with Giemsa stain which shows
  **"Safety pin appearance"/ "Bipolar staining"**

### Donovan body:

- A macrophage filled with bipolar-stained bacteria is called as Donovan body

**L.D bodies are seen in leishmaniasis**

# ENTEROBACTERIACEAE FAMILY PROTEUS

## Proteus 00:00:04

- Pleomorphic
- Motile – By peritrichous flagella
- NLF
- Colonies: fishy or seminal odour
- "Swarming" motility (shown in figure 1)
  - It is also a growth pattern

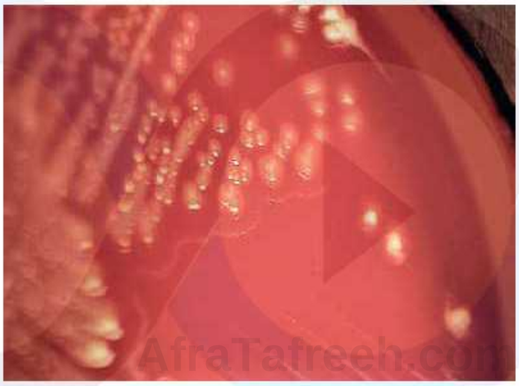


Fig: 1

### Methods to inhibit swarming:

i. Agar concentration increased to 6%
ii. Bile salts
iii. Boric acid
iv. Ethanol
v. Sodium azide
vi. Trimethoprim
vii. Sulfonamides
viii. Chloral hydrate

### Other features of Proteus:

- Produces $H_2S$
- Produces urease enzyme
- Test: PDA test

Phenylalanine $\xrightarrow{\text{Phenyl alanine de-aminase}}$ phenylpyruvic acid

- Proteus – Is a saprophyte
- Also present in gut of humans (coliform)

**Important species:**

- Proteus mirabilis
- Proteus vulgaris
  - P. mirabilis: Indole negative
  - P. vulgaris: Indole positive
- P. mirabilis – more pathogenic

## Diseases 00:09:25

### i. UTI: Urinary Tract Infection

- Characterised by formation of Urinary stones

↓ known as

**Struvite stones/ staghorn calculi/ Magnesium Ammonium Phosphate (MAP) crystals**

- Urea $\xrightarrow{\text{urease}}$ $NH_3 + Co_2$

$$\downarrow \begin{matrix} +Mg^{2+} \\ +PO_4^{3-} \end{matrix}$$

Magnesium-
Ammonium-
Phosphate (MAP) crystals

- $NH_3$ → causes alkalisation of urine
- Alkalisation of urine:
  - Favours stone formation
  - Not much of burning sensation occurs during micturition
- MAP crystals can:
  - Increase in size
  - Block/ obstruct urinary tract
  - Lead to urinary obstruction
  - Septicaemia

ii. Septicaemia in geriatric population

iii. Wound infection of:

Bed sores

Diabetic wounds.

# ENTEROBACTERIACEAE FAMILY-SERRATIA

## Serratia 00:00:08

- Medically important species: Serratia marcescens
- Motile
- Produces red, non-diffusible pigment called as – PRODIGIOSIN
- Prodigiosin – optimally produced at temperature of 30°C
- The organism is a saprophyte in food, water, soil
- Diseases occur in Immunocompromised people
- Diseases caused:
  - Pneumonia
  - UTI
- Pneumonia – characterised by "Pseudo-haemoptysis"
- Pseudo-haemoptysis – Red sputum because of Red pigment And not because of blood

### Hospital stains:

- Are multi-drug resistant (MDR)
- Because of production of Amp C-Beta lactamase
- Can be non-pigmented

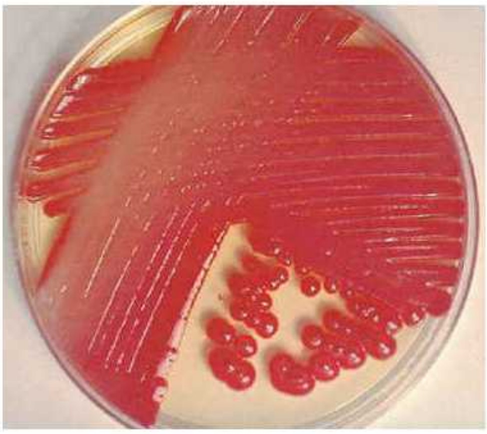


Fig: 1

## Treatment:

- As per report of anti-microbial susceptibility testing
- Intrinsically resistant to polymyxins and nitrofurantoin

# ENTEROBACTERIACEAE

# FAMILY-YERSINIA PESTIS

## Yersinia pestis 00:00:06

- Disease: Plague
- Non-motile
- Non lactose fermenter
- Non haemolytic
- Growth produced on oil/ ghee broth – 'Stalactite Growth'

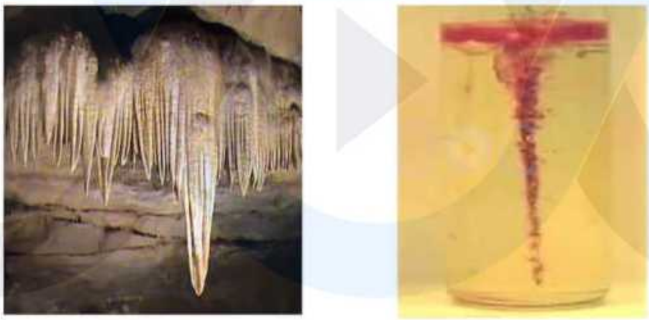


Fig: 1

- Non fastidious – can grow in nutrient broth
- On blood agar:
  - Non haemolytic
  - Brown pigment is formed because of absorption of Hemin
- Gram negative Coccobacillus (oval)
- Capsule: Has $F_1$ Antigen
  - $F_1$ antigen is plasmid encoded
- Capsule: Is antiphagocytic
  - Produced at 37°C
- Optimum growth temperature: 27°C

- **Virulence factors:**
  - i. F1 Antigen
  - ii. Yersinia outer membrane proteins (OMPs)
  - iii. LPS
  - iv. Siderophores
- **Disease:** Plague (Zoonotic disease)
- **Reservoir:** Wild Rodents. (Eg: Field mice, bandicoot etc.)
- **Vector:** Rat flea (Xenopsylla cheopis)

## Modes of infection:

- Most common: Bite of infected rat flea
- Contact with tissues of infected rodents
- Inhalation – pneumonic plague → Can be transmitted from man to man
- Rarely contact with **Human flea (Pulex irritans)**

Natural cycle of plague

- **Domestic cycle**
  - Between Rodents, fleas, humans
- **Wild/ sylvatic cycle**
  - Wild rodents (Independent of humans)

## Types of plague: 00:08:26

| Bubonic | Septicemic | Pneumonic (most dangerous) |
|---|---|---|
| I.P: 2-7days | 2-7days | 1-3days |
| Most common type<br>After flea bites (Usually lower limb)<br>↓<br>Organism enters lymphatics<br>↓<br>Enlarged regional lymph nodes: "Bubos"<br>Most commonly: Inguinal nodes involved | Most commonly occurs as a complication of Bubonic plague or pneumonic plague | Only zoonotic disease which can be transmitted from man to man |
| Bubonic: Not transmitted from man to man (organism blocked in Buboes) | | |

## Diagnosis:

- Specimen:

  Pus from buboes

  Blood

  Sputum

i. **Microscopy:**

- On gram stain: Gram negative coccobacilli with lot of pus cells
- Wayson stain: Bipolar staining/ safety pin appearance

ii. Culture

iii. $F_1$ Ag detection by ELISA

iv. Ab detection by ELISA

- Ab detection by Indirect haemagglutination

v. **Molecular methods:** PCR to detect

- $F_1$ Ag gene
- $F_1$ pesticin gene
- $F_1$ plasminogen activator gene

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## Treatment:

- Streptomycin x 10days
- Gentamicin: Superior, currently recommended

# GRAM POSITIVE SPORE BEARING RODS – BACILLUS

- Spore bearers – Belong to genera:
  - Bacillus
  - Clostridium

## Bacillus 00:00:55

- Either aerobes or facultative anaerobes
- Motile by peritrichous flagella except Bacillus anthracis (Biggest bacterium) which is non-motile
- Common contaminants of culture media
- 2 pathogens:
  - B. anthracis
  - B. cereus
- Spores are non-bulging
- Gram positive rods

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## Bacillus anthracis 00:03:42

- Biggest bacterium: Size: 4-8 x 1-1.5 µm
- Non-motile
- 1st microbe seen under the microscope by Pollender
- 1st bacterium grown on solid media by Sir Robert Koch
- 1st bacterium where spores were discovered by Sir Robert Koch

- 1st bacterial disease for which a vaccine was devised by **Sir Louis Pasteur**
- 1st bacterial disease for which a serological test was devised known as **Ascoli's Thermoprecipitin Test**
- 1st infectious disease shown to be **transmitted** via **infected blood**

## Bacillus anthracis:

- Non-fastidious
- Selective media: PLET medium which has
  - Polymyxin B
  - Lysozyme
  - EDTA
  - Thallous acetate
- Non-haemolytic on blood agar
- Cultural appearances:

1. Frosted glass appearance: Figure 1 shows non-haemolytic colonies on blood agar which look like frosted glass.

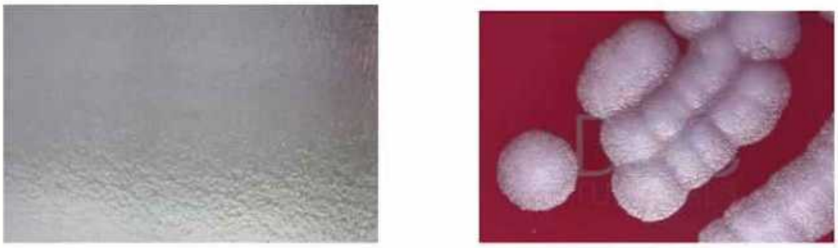


Fig: 1

2. Inverted fir-tree appearance on gelatin liquefaction:

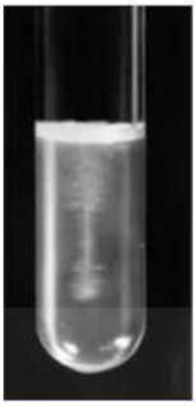


Fig: 2

3. Medusa head appearance:

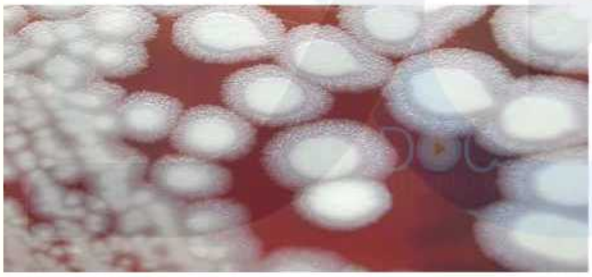


Fig: 3

## Microscopic appearances:

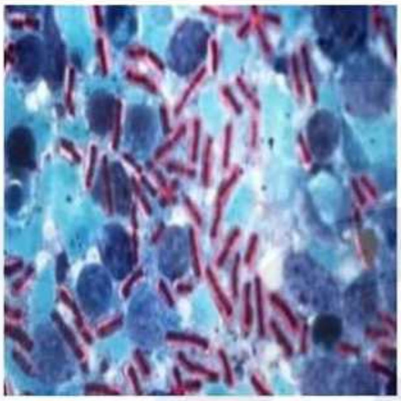
1. Box-car shaped appearance
2. Bamboo-stick appearance
3. String of pearls appearance seen on culture medium containing penicillin

## Virulence factors:

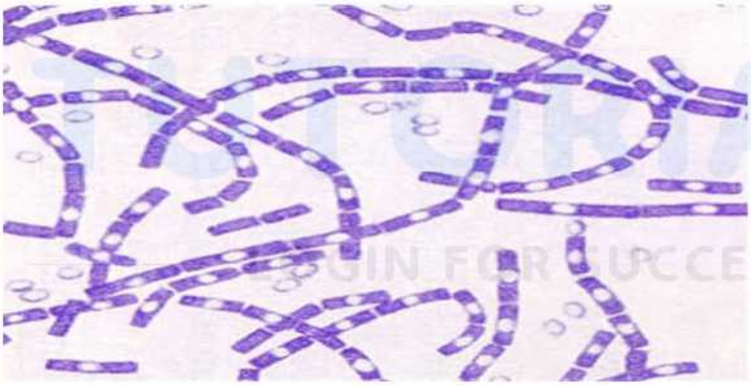
1. Capsule:
   - Protein capsule – polymer of d-glutamic acid Hence called as "Poly-d-glutamate"
   - Anti-phagocytic

- Plasmid encoded ($PXO_2$)

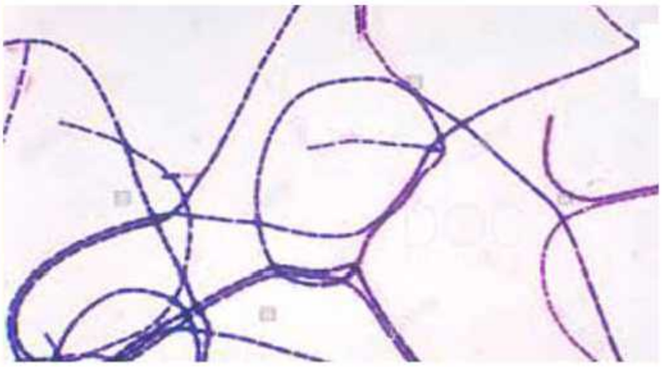
  Capsule can be shown by Mcfadyean's reaction using polychrome methylene blue where capsule looks like purplish amorphous material around body of bacteria (figure 4)



Mcfadyean reaction Fig: 4



Gram stain of Bacillus anthracis Fig: 5



Box car appearance on Gram stain Fig: 6

2.Toxin:

**Exotoxin:3 components**

- I: Edema factor
- II: Protective factor
- III: Lethal factor

- *Gene for the exotoxin is also present on a plasmid (PXO1)*

**Factor-II:** Acts as binding subunit (binds to host cell receptor)

**Once Factor-II binds to host cell receptor:**

- **Factor-I enters host cell ↓ Edema**
- **Factor-III Enters host cell ↓ Cell death**

- Factor-II: called as "Protective Ag" because antibodies to it are protective against disease
  - Disease: Anthrax – It is a disease of herbivorous animals

**Human are relatively resistant to anthrax**

- For humans – Anthrax is a zoonotic disease

### Modes of infection:

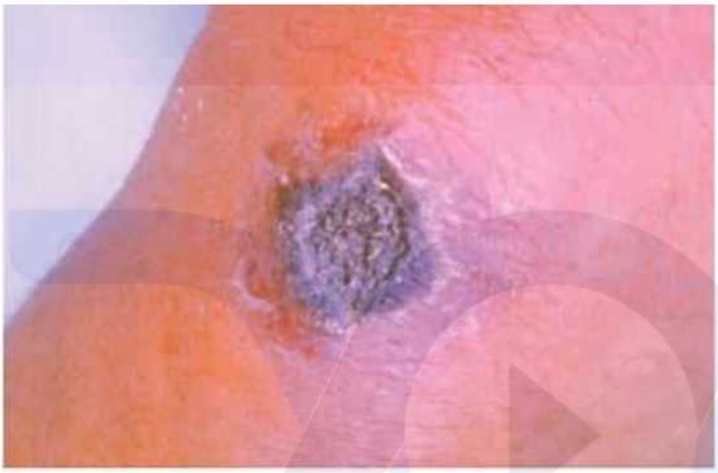
- Inoculation:
  - Cutaneous anthrax/ hide porter's disease develops
  - Most common type of anthrax
  - Least dangerous
- Ingestion:
  - By ingestion of meat of infected animal
  - Intestinal anthrax develops (necrotic lesions in the intestine)
  - Rarest form of anthrax
  - 2nd most dangerous
- Inhalation:
  - Because of inhalation of spores, inhalational anthrax/wool sorter's disease
  - 2nd most common type
  - Most dangerous

### Cutaneous anthrax:

- Least dangerous
- Lesion: Malignant pustule

"Central black necrotic eschar surrounded by oedema"

- Toxaemia is present



Black eschar Fig: 7

### Intestinal anthrax

- 2nd most dangerous
- Necrotic lesions present in intestine
- Oropharyngeal anthrax is also reported

### Wool sorter's disease/ inhalational anthrax:

- Most dangerous
- Characteristic feature is Haemorrhagic Mediastinitis
- On chest x-ray: symmetrical widening of mediastinum

### Treatment:

- DOC: Ciprofloxacin
- B. anthracis is category- 'A' Bioterrorism agent
- "Poor terrorist's" weapon

## Bacillus cereus 00:26:11

- Causes food poisoning

Food poisoning

| Emetic type | Diarrhoeal type |
|---|---|
| Main clinical feature: Vomiting | Main clinical feature: Diarrhoea |
| I.P: 1-6 hours<br>Median I.P ≈ 1hour | I.P: 8-16 hours |
| - It is because of preformed toxin<br>- Purely toxic type of food poisoning | - It is due to intake of bacteria which then produce an enterotoxin in gut (≈ to toxin of ETEC)<br>- Toxico-infective type of food poisoning |
| Foods: Chinese fried rice<br>Reheated foods eg. Buffets | Foods: Meat & meat products, vegetables and cereals |
| Serotypes responsible for emetic type – 1,3,5 | Serotypes responsible for diarrheal type – 2,6,8,9,10,12 |

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- Selective medium for B.cereus: MYP agar (Mannitol egg yolk polymyxin-B)

# GRAM POSITIVE NON-SPORING RODS

# -LISTERIA MONOCYTOGENES

## Listeria monocytogenes 00:00:04

- Gram positive rods/coccobacilli
- Only G+ organism having LPS/endotoxin
- Motile: Tumbling motility

**Differential motility**

**Motile at 25°** **Non motile at 37°**

- Sir Joseph Lister → worked on Listeria monocytogenes
- Psychrotropic – Grows at both 37° and low temperature (4°C)
- Saprophyte
- Produces listeriolysin 'O' (LLO) – a β-Hemolysin
  ↓
  Causes β-Hemolysis on blood agar
- Anton's test (similar to Sereny's test)
- Listeria also shows positive CAMP TEST
- Catalase positive

## Diseases 00:07:00

### Gastroenteritis:

- IP: 16-18 hours
- Foods: Milk & milk products specially soft cheese
- Bacteraemia develops, but no symptoms in immunocompetent individuals

### Infection during pregnancy:

- Still birth
- Abortion
- Premature rupture of membranes – premature labour

### Infecting occurring near term:

- Baby develops **Granulomatosis infantisepticum** characterised by – Granuloma formation in liver, spleen etc

> **Granuloma formation occurs in cases of infection by facultative intracellular organisms**

- L. monocytogenes is a facultative intracellular organism

### Infection in Neonates:

- Neonatal meningitis

### Meningitis in geriatric population:

L. monocytogenes causes meningitis in geriatric population

NOTE: In geriatric population, MCC of Meningitis- Gram negative enteric bacilli

### Meningitis in immunocompromised people:

- Eg: Transplant recipients
  - Steroid therapy recipients
  - Alcoholics

- **L. monocytogenes** – spreads from 1-cell to another by forming **Actin rockets**

## Host cell receptor:

- Host cell receptor is internalin – A, B
- Listeria resists killing inside phagolysosome via virulence factors like Listeriolysin-O, phospholipases

↓

The organism causes polymerisation of host cell actin filaments by expressing a protein – **Act A**

- Since, it is a facultative intracellular organism, L. monocytogenes can evade antibodies
- Mode of infection: Food-borne

## Treatment:

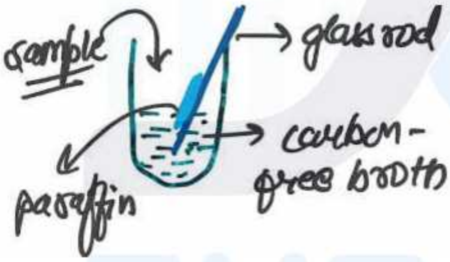
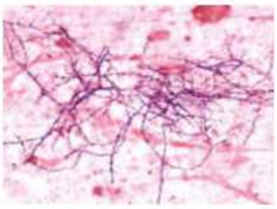
- Ampicillin + Gentamicin
  - Cephalosporins are not used

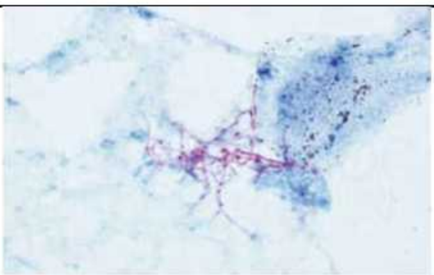
# GRAM POSITIVE FILAMENTOUS BACTERIA – NOCARDIA AND ACTINOMYCES

## Gram positive filamentous bacteria 00:00:10

- Nocardia
- Actinomyces
  - Both have prokaryotic ribosomes

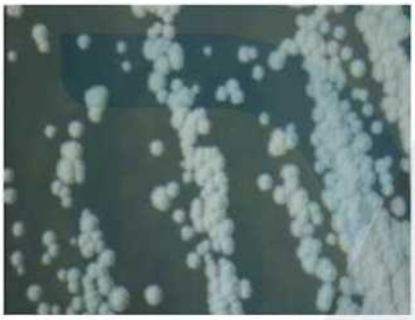
| Nocardia | Actinomyces |
|---|---|
| Aerobic organism | Anaerobic organism |
| Partially acid fast (1% $H_2So_4$ – Decolouriser) | Non-Acid fast |
| Not a commensal | It is a commensal in oral cavity, gut, genitourinary tract in humans |
| Exogenous infection (It is a saprophyte ) | Endogenous infection |
| Causes disease in immunocompromised people | Causes disease in Immunocompetent people |
|  | Most common species: Actinomyces israelli |
| **Types of Nocardiosis:** | **Types of Actinomycosis** |
| 1.Pulmonary nocardiosis: | 1.Cervicofacial actinomycosis/ Woody jaw |

| | |
|---|---|
| - Productive cough is present without lymphadenopathy<br>2. Brain abscess<br>NOTE: For culture of Nocardia:<br>Paraffin bait technique:<br>- Nocardia uses paraffin as the sole source of carbon<br><br> <br>• Growth can be subcultured on other media. Eg: SDA<br><br>↓<br>Delicate beaded filaments of Nocardia on gram stain | - Very hard swelling of submandibular region<br>Risk factors: Oral procedures<br>Eg: Dental extraction<br>2. Thoracic actinomycosis<br>3. Abdominal actinomycosis<br>4. Pelvic actinomycosis<br>Risk factors: IUCD use<br>• Colonies of actinomycosis are:<br>- Molar tooth colonies/spider like colonies |



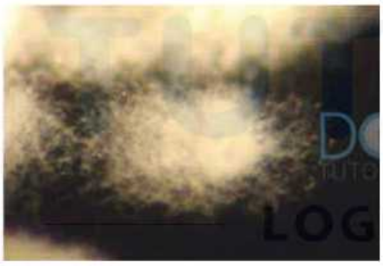
↓

- Nocardia Acid-fast stain (Partially acid fast )



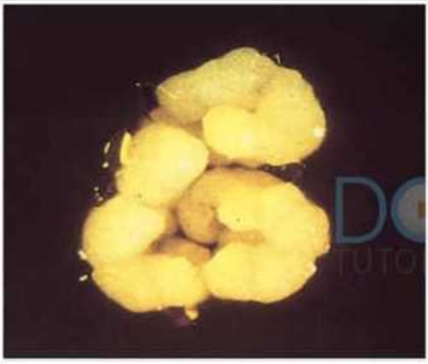
↓

Colonies of Nocardia



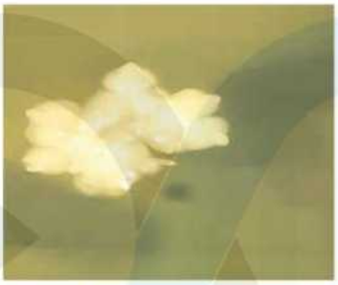
↓

Aerial hyphae of Nocardia

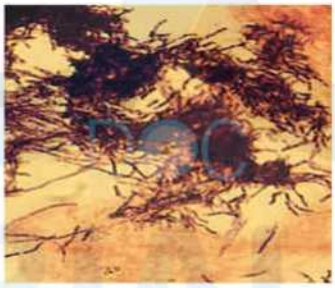


↓

Molar tooth appearance of Actinomyces



1.



2.

1. Macroscopic colony
2. Gram stain

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## Treatment

| S | N | A | P |
|---|---|---|---|
| Sulfonamides- Nocardia | | Actinomyces- Penicillin | |

# GRAM NEGATIVE COCCI – NEISSERIA

## Neisseria 00:00:10

- Gram negative diplococci
- Fastidious organisms
- Strict aerobes
- Catalase positive
- Oxidase positive
- Some species are commensals in oral cavity and genitourinary tract
- Non-motile
- 2 important species are pathogens
  - N. meningitidis /Meningococcus
  - N. gonorrhoeae /Gonococcus

| **N. meningitidis** | **N. gonorrhoeae** |
|---|---|
| • Planoconvex cocci | • Kidney/ bean shaped cocci |
| • Capsulated organisms | • Non-capsulated organisms |
| • Utilises both glucose and maltose | • Utilises only glucose |
| • Mode of infection: through respiratory route | • Mode of infection: Sexual intercourse |
| • Organism presents in nasopharynx | |
| • Strict human pathogen | • Strict human pathogen |
| • Does not produce β-lactamase | • Produces β-lactamase |
| • Drug resistance – Less common | • Drug resistance – More common |
| • Virulence factor: Capsule | • Virulence factor: Pili |

## Meningococcus 00:07:46

- Site of carriage – Nasopharynx

**From Nasopharynx**

Haematogenous route
(More common)
↓
To reach meninges

Through cribriform
plate via perineural
sheath of olfactory
nerve (less common)
↓
Reaches CNS

- Disease causing serotypes – A, B, C, W-135, Y
- Serotype – A – More common in India
- Serotype – B – More common in West
- Diseases:

  1.Meningitis – in overcrowded areas

  2.Meningococcaemia/Purpura fulminans:

    Triad:
    - Purpuric rash
    - Shock
    - Meningitis

  3.Waterhouse friderichsen syndrome:
    - Patient dies in 3-5 hours
    - On autopsy: Bilateral adrenal haemorrhage

### Diagnosis:

- Sample:
  - CSF
  - Blood

    1.Gram strain:

- Lot of neutrophils with intracellular & extracellular gram-negative diplococci

2. Culture:
    - Blood agar
    - Chocolate agar

    ] Enriched media

- Modified Thayer martin medium – selective medium
    - One should incubate the culture plates in 5-10% $CO_2$ because they are capnophiles

**Treatment:**

- DOC for treatment & prophylaxis – Ceftriaxone

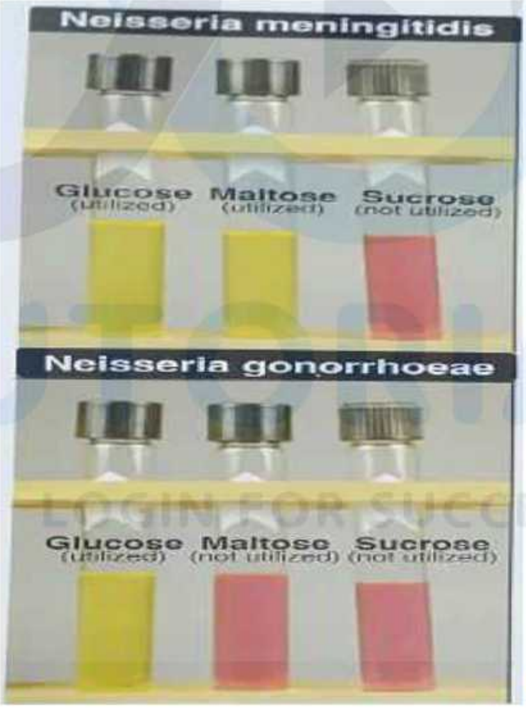
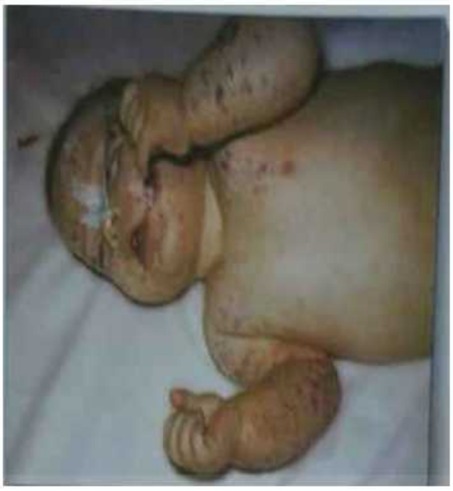
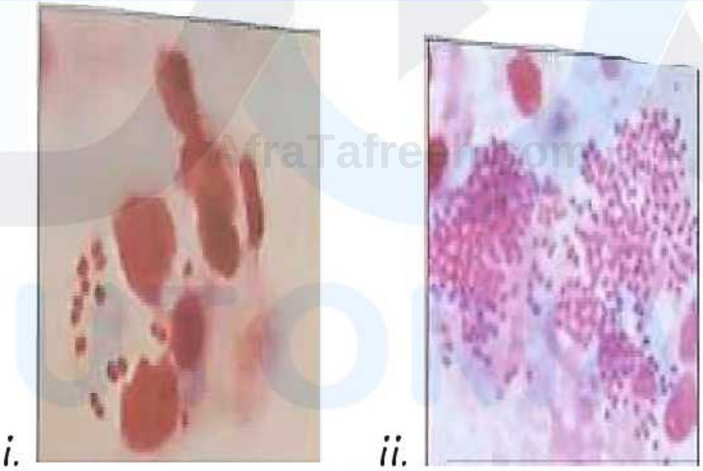

Neisseria meningitidis
Glucose (utilized)
Maltose (utilized)
Sucrose (not utilized)
Neisseria gonorrhoeae
Glucose (utilized)
Maltose (not utilized)
Sucrose (not utilized)

Fig: 1

Neisseria meningitidis produces acid from oxidation of glucose and maltose, but not from sucrose. The acid turns the pH indicator phenol red from red to yellow



Petechial and/or purpuric rash and neck extension characteristic of meningococcal meningitis Fig: 2



i. ii.

Fig: 3

i. Neisseria gonorrhoea in clumps within polymorphonuclear leukocytes in urethral exudate.
ii. Neisseria meningitidis in cerebrospinal fluid of patient with meningitis.

## Neisseria gonorrhoeae/ Gonococcus 00:17:28

- Disease: Gonorrhoea – a sexually transmitted infection

- I.P: 3-5days
- Some strains of Gonococcus are motile – Twitching motility
- Virulence factor – PILI
  - Pili – help the organism to attach to urethral & endocervical epithelium
  - Typing of Gonococcus is done based on the amount/distribution of pili, known as Kellog's typing

### Clinical features:

| Males | Females |
|---|---|
| • Urethritis – urethral discharge<br>• Usually, symptomatic | • Endocervicitis<br>• Usually asymptomatic (Reservoir of Infection)<br>• Vaginal infection does not occur because stratified squamous epithelium is resistant to Gonococcal infection |
| **Complications** | |
| 1.Periurethral abscess<br>2.Watercan perineum (Multiple discharging sinuses in perineal region)<br>3.Disseminated Gonococcal Infection (DGI):<br>Tenosynovitis<br>Rash | 1.PID – Gonococcus is an important cause of acute PID<br>2.Secondary infertility<br>3.Ectopic pregnancy<br>4.Fitz Hugh Curtis syndrome:<br>- Perihepatitis<br>- Periappendicitis<br>5.DGI |

| | |
|---|---|
| Septic arthritis<br>↓<br>Gonococcus: Most common cause of septic arthritis in sexually active adults | 6.Ophthalmia neonatorum |

- DGI (Disseminated Gonococcal Infection) – seen in people with deficiency of late complement proteins ($C_5$ to $C_9$)

NGU (Non-Gonococcal urethritis
>> Gonococcal Urethritis (GU)

- Complications are same in both Gonococcal and non-Gonococcal urethritis (NGU)
- But complications are more common in GU except:

Fitz Hugh Curtis syndrome
↓
Most common cause is
Chlamydia trachomatis (D to K)

Diagnosis:

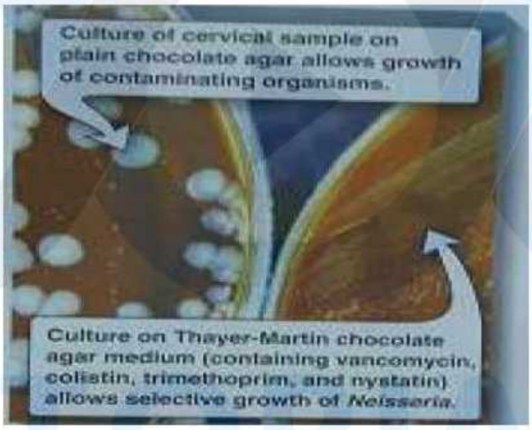
- Sample:
  i. Males:
    - Best sample– urethral discharge. If discharge is absent, milk the urethra & express discharge. If this fails, periurethral swab is collected
  ii. Females:
    - Endocervical swab

1. Gram strain (GS):
   - Lot of neutrophils, both intra and extracellular gram-negative diplococci

If lot of neutrophils present, but no organism on GS: **Think of Non-Gonococcal Urethritis**

2. Culture:
   - On blood agar
   - Chocolate agar

     **Enriched media**
   - Modified Thayer martin media – Selective media



Culture of cervical sample on plain chocolate agar allows growth of contaminating organisms.

Culture on Thayer-Martin chocolate agar medium (containing vancomycin, colistin, trimethoprim, and nystatin) allows selective growth of *Neisseria*.

Fig: 4

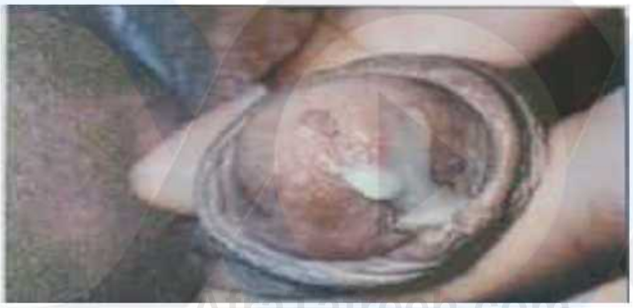
Left: Mixed growth on plain chocolate agar. Right: Pure culture on ThayerMartin chocolate agar medium

- Modified Thayer martin medium is chocolate agar, it has
  - **Hemin**
  - Vancomycin (inhibits Gram positive organisms)
  - Colistin (inhibits Gram negative organisms)
  - Nystatin (Antifungal)
  - Trimethoprim (inhibits swarming of proteus)

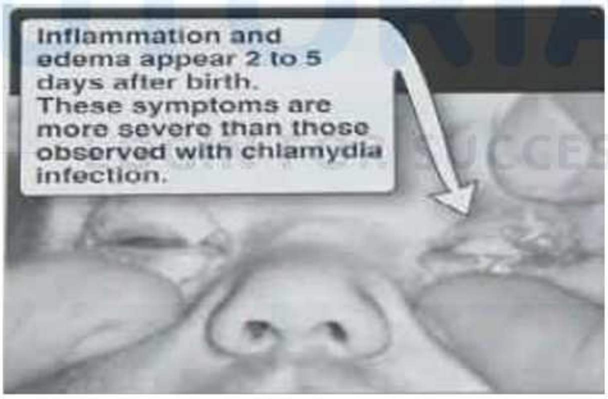
- Transport media – for both Gonococcus & Meningococcus:
  i. Amies medium
  ii. Stuart medium
- Transport media maintains viability of organisms

## Treatment:

- DOC for Gonococcal infections: Ceftriaxone
- Pili of Gonococcus: Show antigenic variation



Urethral discharge of gonorrhea Fig: 5



Inflammation and edema appear 2 to 5 days after birth. These symptoms are more severe than those observed with chlamydia infection.

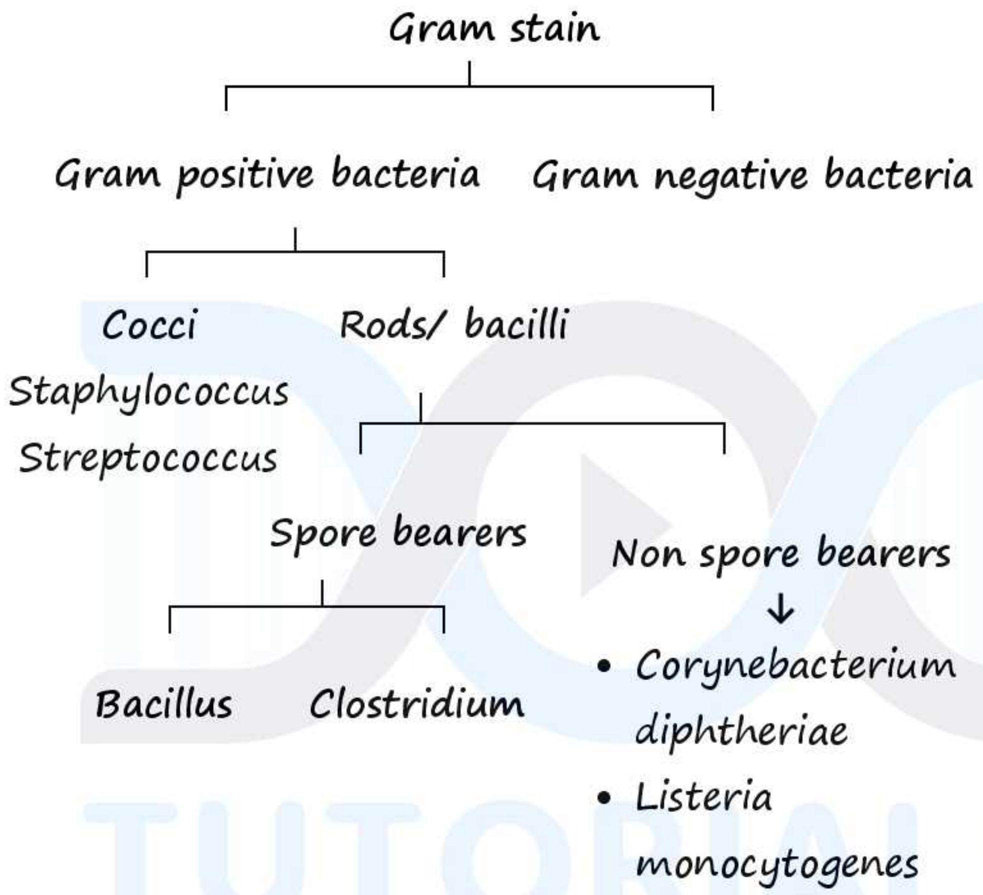
Gonococcal ophthalmia neonatorum

Fig: 6

# GRAM POSITIVE BACTERIA:

# STAPHYLOCOCCUS PART - I

## Classification of gram-positive bacteria 00:00:11


Gram stain
Gram positive bacteria
Gram negative bacteria
Cocci
Staphylococcus
Streptococcus
Rods/ bacilli
Spore bearers
Non spore bearers
Bacillus
Clostridium
• Corynebacterium diphtheriae
• Listeria monocytogenes

## Genus Staphylococcus 00:02:36

- These are gram positive cocci, appear as bunch of grapes → Grape like clusters
- Bunch of grapes appearance is because of cell division taking place in 3 planes
- Non-fastidious (can grow on simple/ basal culture media)
  - Eg: Nutrient agar
- Non-motile

- Facultative anaerobes
- Catalase positive
- Oxidase negative

**Based on Coagulase enzyme production**

Coagulase Positive (Coagulase-produced)
↓
Coagulase positive staphylococci
Eg: S. aureus – most important pathogen

Coagulase Negative (Coagulase – not produced)
↓
Coagulase negative staphylococci (CoNS)
- **S. epidermidis**
- **S. saprophyticus**

## S. aureus 00:07:46

Culture media:

### i. Nutrient agar:

- Colonies are of 2-3mm diameter
  - "Pin head colonies" – Golden yellow coloured
- Golden yellow colour is because of golden yellow pigment called "Staphyloxanthin (carotenoid pigment)
- If confluent growth is present –called as 'Oil paint appearance '

### ii. Blood agar:

- An enriched medium

- Same kind of colonies produced as on nutrient agar

  +

  β-hemolytic colonies

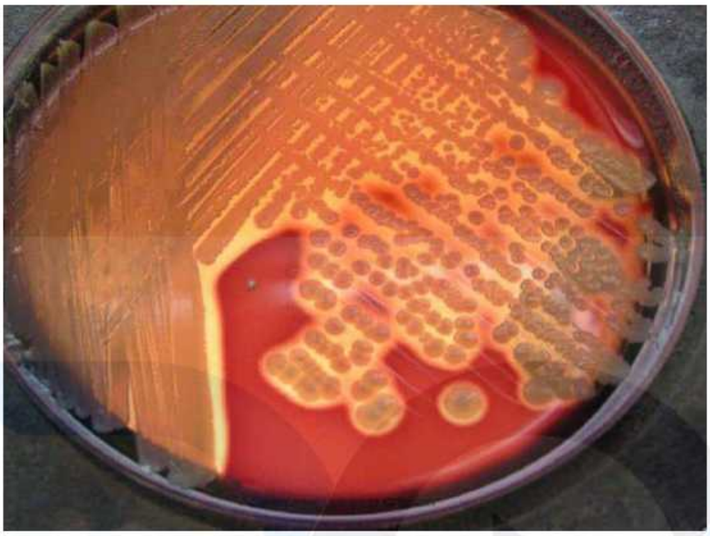
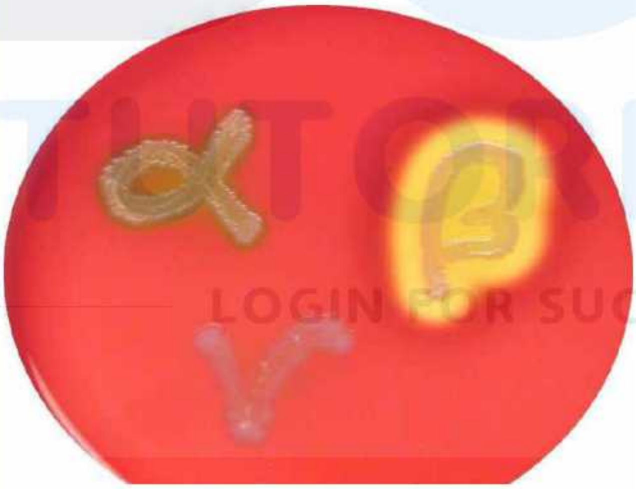


Fig: 1

Golden yellow haemolytic colonies of S. aureus



Types of haemolysis

Fig: 2

- β-haemolysis – complete haemolysis (bright yellowish)
- α-hemolysis – partial haemolysis (greenish discoloration due to biliverdin)

- γ-hemolysis – no haemolysis

- Haemolysis on blood agar plate: Interpreted in transmitted light

### iii. Milk agar:

- Nutrient agar + milk
- Pigment production is better

### iv. Salt milk agar:

- Milk agar + 7.5% salt

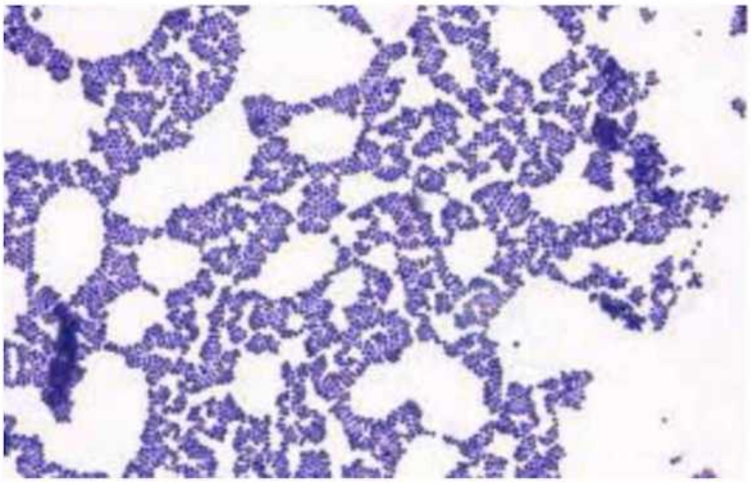
All species of Staphylococcus genus can grow in the presence of 7.5% salt

### v. Mannitol Salt Agar (MSA): Selective and differential medium:

- Selective medium: Allows growth of Staphylococcus genus
- Differential medium: Helps to differentiate between different species of Staphylococcus based on mannitol fermentation.

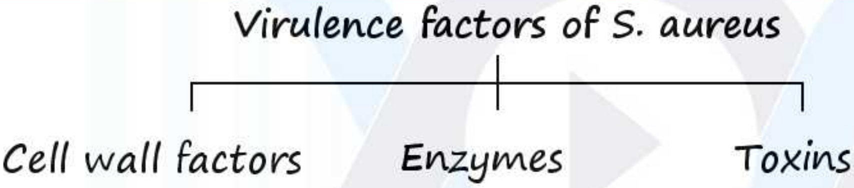
On MSA: S. aureus → yellow colonies ∵ of mannitol fermentation

### vi. Ludlam's medium: Has lithium salts in it



Gram stain of Staphylococcus Fig: 3

## Virulence Factors 00:21:46



Virulence factors of S. aureus
Cell wall factors
Enzymes
Toxins

### I. Cell Wall Factors:

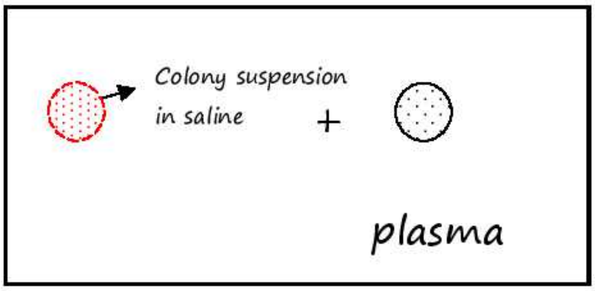
1. Lipoteichoic acid:
    - Helps in attachment to host cells
    - Plays a role in septic shock
2. Protein – A: It is antiphagocytic
    - Serves as binding factor for $F_c$ part of IgG
    - Anti-complement activity ($\because$ It binds to $F_c$ part)

**$F_c$ part of Ab binds to complement**

- protein A used in Conglutination test
- Example of a strain of S. aureus having protein A is **Cowan strain**

3. Bound coagulase/Clumping Factor:

- It is a receptor for fibrinogen
- Detected by slide coagulase test:



Visible clumping seen, if colonies are of Staph aureus

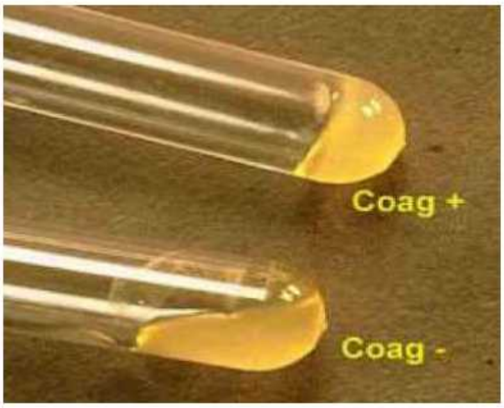
## II. Enzymes:

**Most important virulence factor of S. aureus is free coagulase/ coagulase enzyme**

1. Free coagulase/Enzymatic coagulase converts:

   Prothrombin → Thrombin
   ↓
   Fibrinogen → fibrin clot (forms wall around the site of infection)

- Due to fibrin clot formation, S. aureus causes localised pyogenic/suppurative infection
- The coagulase enzyme is detected by tube-coagulase test



Tube coagulase test Fig: 5

2.DNAase

3.Thermostable nuclease

4.Phosphatase

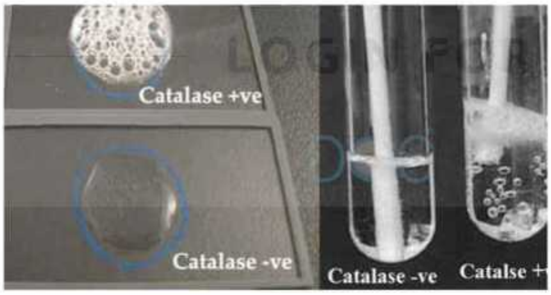
5.Hyaluronidase

6.Staphylokinase

Catalase enzyme: Detected by Catalase test:

↓

$H_2O \rightarrow H_2O + O_2$


Catalase +ve
Catalase -ve
Catalase -ve
Catalse +

Catalase test Fig: 6

## Mnemonic Related to Staphylococcus:

Staphylokinase
Thermostable nuclease
protein – A

Phosphatase

Hyalurondiase

Yellow (golden colonies)

Leukocidin

O

Catalase positive

O

Cogulase positive – S.aureus

Cogulase negative – CONS

Urease positive

S

### III. Toxins of S. aureus:

- Haemolysins:

  α – hemolysin/paradoxical toxin:

  - It gets inactivated when heated to 60-70°C. But when temperature rises to 100°C, it gets reactivated
  - At 60-70°C – Toxin binds with an inactivator (which is heat labile)
  - At 100° C – The heat labile inactivator itself gets inactivated.

- β- hemolysin/Sphingomyelinase/Hot-cold haemolysin:

  - Sphingomyelin: It is present in cell membrane of sheep RBCs.
  - β-haemolysin causes lysis of sheep RBCs

- "Hot-cold haemolysin" – Called so because the toxin binds to RBCs at a higher temperature but actual haemolysis occurs when the suspension is cool.
- β – hemolysin – Is used in **CAMP test**

- Leukocidin/Panton-valentine toxin:
  - Made of 2 components which synergistically act to insert holes in the cell membrane of WBCs
  - Is an example of SYNERGOHYMENOTROPIC TOXIN – bicomponent membrane – active toxins
  - γ-hemolysin → It targets RBCs – is also an example of synergohymenotropic toxin
  - δ – haemolysin: Has detergent-like action on RBCs, WBCs, platelets, macrophages
- Epidermolytic toxin/Exfoliatin:
  - Acts as a protease, causes separation of epidermis at the granuler cell layer because it cleaves desmoglein of desmosomes
  - Associated with **Staphylococcal Scalded Skin Syndrome (SSSS)**
  - It is a superantigen
- Enterotoxins:
  - They are exotoxins
  - Exquisitely heat stable – can withstand 100°C for 30 minutes.

    Disease: Food poisoning

- They are superantigens

  Most common site of carriage of S. aureus is anterior nares

  2nd most common site of carriage of S. aureus is skin

## Clinical Features of food poisoning by S. aureus:

- Vomiting due to vagal stimulation
- Sometimes diarrhoea (watery, non-bloody)
- Types of enterotoxins: A to G (except F)
- Self-limiting disease
- I.P: 1-6 hours
- Occurs because of preformed/Intradietetic toxin
- These enterotoxins are superantigens

### TSST-1/Toxic shock syndrome toxin-1: -

TSST-1 is the new name for enterotoxin-F

- It is a superantigen
- Disease: Toxic shock syndrome (TSS)

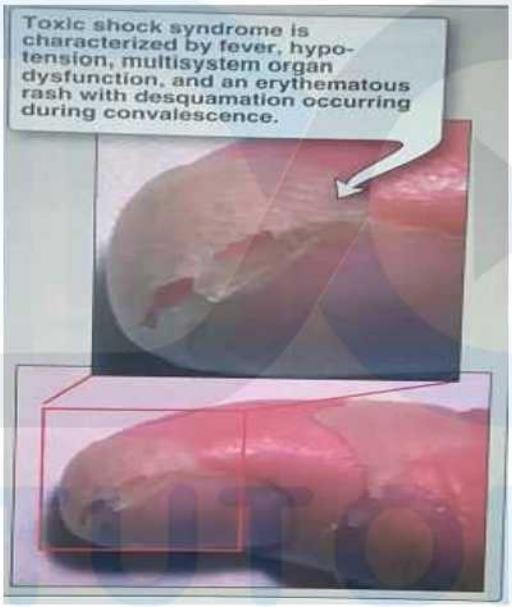
# STAPHYLOCOCCUS PART-II

## Diseases caused by Staphylococcus aureus

00:00:53

Disease caused by Staphylococcus aureus

- Pyogenic/ suppurative
- Toxin mediated diseases



Toxic shock syndrome is characterized by fever, hypotension, multisystem organ dysfunction, and an erythematous rash with desquamation occurring during convalescence.

Toxic shock syndrome Fig: 1

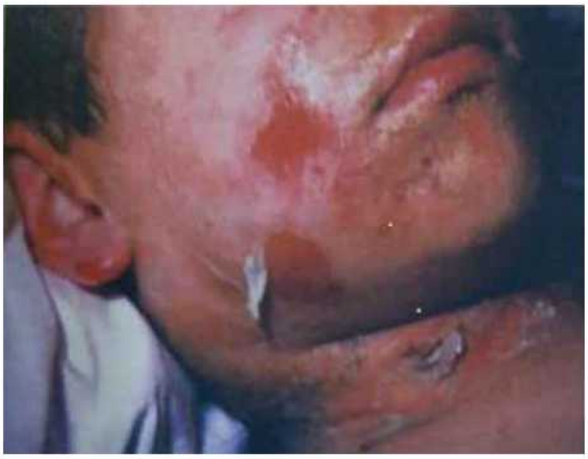


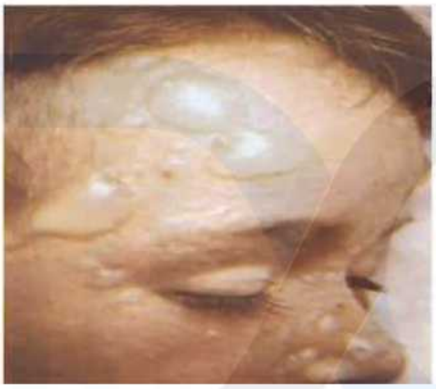
Fig: 2

Staphylococcal scalded skin syndrome

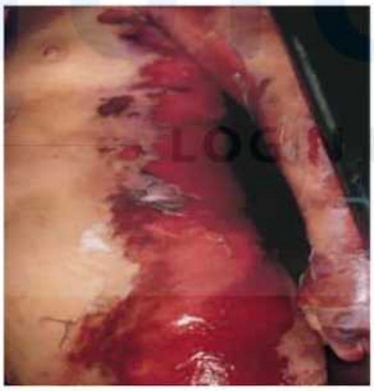
## Pyogenic/ suppurative diseases 00:01:18

- Pyoderma: Most Common Cause (MCC) is S. aureus
- Impetigo

  NOTE: MCC of impetigo is Group A Streptococcus (GAS)
- Bullous impetigo (Caused only by Staphylococcus aureus, not GAS)



Bullous impetigo Fig: 3



Toxic shock syndrome Fig: 4

- Folliculitis – superficial infection of hair follicle
- Furuncle – Deep infection of hair follicle
- Carbuncle – Multiple furuncles

- Cellulitis

  NOTE: MCC of cellulitis is GAS
- Breast abscess (MCC) – usually seen in lactating women
- MCC of acute osteomyelitis

**Most common cause of acute osteomyelitis in sickle cell anemia patients is Salmonella**

- Acute endocarditis (MCC) – usually occurs in intravenous drug abusers
  - Usually, right sided valves are involved
- Hospital-acquired/Nosocomial pneumonia
- Surgical site infection
- Community acquired pneumonia (CAP):
  - Special variety of CAP known as "Necrotising pneumonia"

    ↓

    Caused by CA-MRSA strains which are leucocidin producers

## Treatment for S. aureus infections:

- Drug of Choice (DOC): Penicillin (if organism is sensitive to it)
- Resistance is common and is because of β-lactamase/penicillinase present on plasmid and can be transmitted from resistant strain to sensitive strain via phages (Transduction)

- Subsequently, penicillinase-resistant penicillins were developed
  - Eg: **Methicillin**, cloxacillin, dicloxacillin, naficillin

↓ Then resistance developed to these drugs

Such S. aureus strains came to be known as **Methicillin resistant**

**S.aureus (MRSA)**

- MRSA strains express altered penicillin binding proteins/ $PBP_{2a}$/ PBP2
  - Gene for these proteins: Mec A gene which is present on bacterial chromosome
- DOC for MRSA: Vancomycin
- Cephalosporins effective against MRSA strains:
  - Ceftaroline
  - Ceftibuten
  - Ceftobipirole
- Vancomycin resistant S. aureus/VRSA:
  - DOC: Linezolid
  - Synercid (synergistic combination of quinupristin & dalfopristin)

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## Coagulase Negative Staphylococcus (CoNS)

00:13:54

Coagulase Negative Staphylococcus (CoNS)

- S. epidermidis
- S. saprophyticus

### Staphylococcus epidermidis:

- Skin commensal
- Non-haemolytic
- Non-pigmented
- Does not ferment mannitol

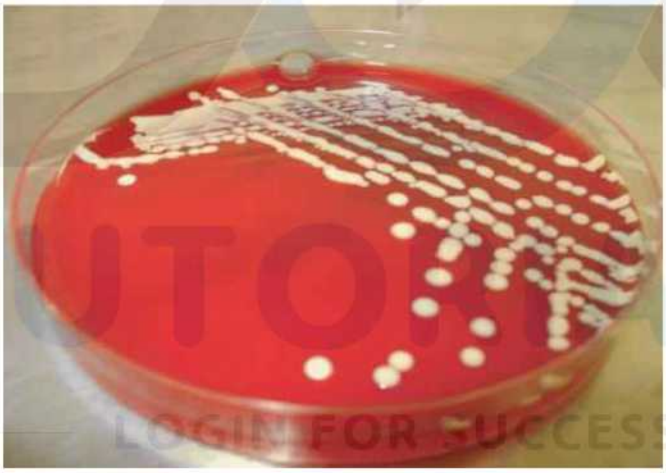
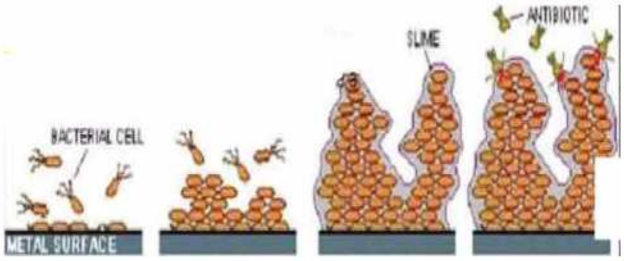


Fig: 5

White, non-haemolytic colonies of S.epidermidis

- Causes disease in the setting of prosthetic devices
  - Eg. Implants, catheters, cannulas, artificial heart valves
- Slime is formed in the presence of prosthetic devices
- Slime is impenetrable by antibiotics, antibodies and complement

- These multiplying organisms in the polysaccharide (slime) substance is known as biofilm



Formation of biofilm Fig: 6

- S. epidermidis: MCC of nosocomial bacteraemia
- S. epidermidis: MCC of endophthalmitis following cataract surgery
- S. epidermidis: An important cause of peritonitis in Continuous Ambulatory Peritoneal Dialysis (CAPD) patients

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## S. saprophyticus 00:20:16

- Causes UTI in sexually active females

**Most common gram-positive coccus causing UTI in sexually active females**

- Otherwise MCC of UTI in sexually active females: E.coli

### Novobiocin Susceptibility testing:

- MOA: Inhibition of DNA gyrase (similar to FQs)

Novobiocin susceptibility testing

S. epidermidis ↓ Sensitive

S. saprophyticus ↓ Resistant

# STREPTOCOCCUS: PART - II

## Diseases caused by Group A Streptococcus / Streptococcus pyogenes 00:00:10

Disease caused by Group Streptococcus/ Streptococcus pyogenes

**Suppurative/Pyogenic infections**

- Pyoderma
- Impetigo: MCC
- Cellulitis: MCC
- Necrotizing fasciitis
- Sore throat

**Non-suppurative immunological sequelae:**

i. Acute rheumatic fever (ARF)

ii. Post streptococcal glomerulonephritis Glomerulonephritis (PSGN)

GAS: Most common bacterial cause of sore

Overall MCC of sore throat are viruses

### Complications of sore throat:

- Ludwig's angina
- Peritonsillar abscess
- Rash causing illnesses:

- Scarlet fever – Rash, sore throat, Strawberry tongue
- Erysipelas
- Toxic shock syndrome

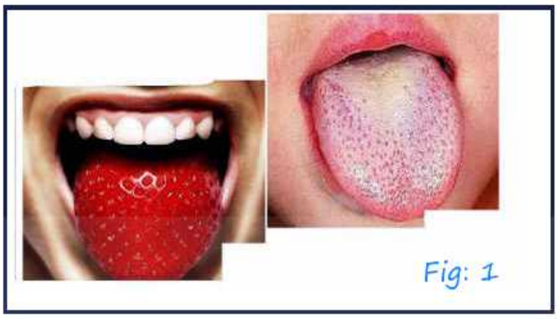


Fig: 1

Strawberry tongue

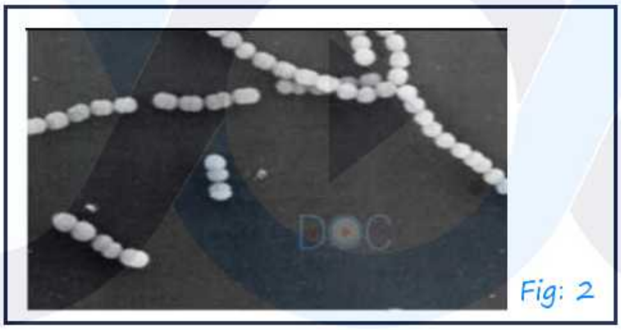


Fig: 2

Scanning electron microscope showing chain of streptococci

## Erysipelas

- Superficial lymphangitis characterized by a well demarcated rash
- Common on face & limbs

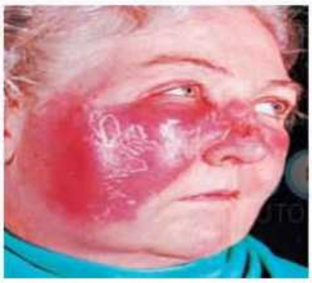


Fig: 3

Erysipelas – Superficial dermis

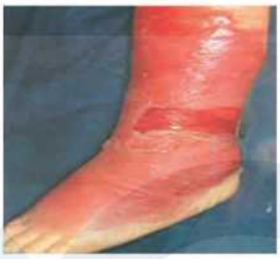


Fig: 4

Acute stage of erysipelas

- Toxic shock syndrome (TSS): Features are similar to those of staphylococcal TSS except that rash is less common

## Acute rheumatic fever 00:07:07

- Occurs 2-4weeks after an attack of sore throat by GAS and not after skin infection
- Antibodies against the bacteria start attacking host tissues because of molecular mimicry

**Molecular mimicry:**

- In S. pyogenes, M. proteins are present on its surface. Human myocardial cells possess a myocardial protein which is structurally similar to S. pyogenes

- In S. pyogenes infection, Ab produced against M-protein also attack myocardial proteins → Myocarditis occurs
- ARF - Clinical features: Fever
  - Myocarditis
  - Subcutaneous nodules
  - Migratory polyarthritis
  - Chorea
  - Endocarditis

**ARF: is an example of Type-II hypersensitivity**

| Streptococcal antigen | Human antigen |
|---|---|
| Cell wall M protein (of serotypes M1, M5, M6, and M19) | Myocardium (Tropomyosin and myosin) |
| Cell wall C carbohydrate | Cardiac valves |
| Cytoplasmic membrane | Glomerular vascular intima |
| Peptidoglycan | Skin antigen |
| Hyaluronic acid | Synovial fluid |

## Post streptococcal Glomerulonephritis 00:13:32

- Occurs 2-4weeks after skin infection by group A Streptococcus-Most commonly, or following sore throat (Less commonly)
- Ab against the bacteria combine with bacteria

↓

Form Immune complexes

↓

Circulate in the blood

↓

Reach kidneys where they are filtered by glomeruli, and tend to deposited in glomeruli & activate complement

↓

Glomerulonephritis

### Clinical features:

- Oliguria
- Edema
- Hematuria

Prognosis: Good

**Unlike ARF – Prognosis is good**

**In ARF – Lifelong penicillin is needed**

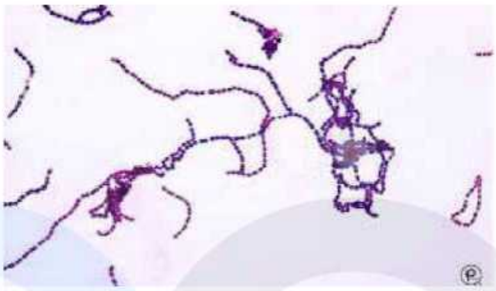
**PSGN – Eg: of type – III hypersensitivity reaction**

## Post streptococcal Glomerulonephritis 00:13:32

- DOC: Penicillin

  ↓ If allergy

  Macrolides



Gram stain of Streptococci Fig: 5

## Group-B Streptococcus 00:18:04

- Beta-hemolytic
- Bovine mastitis
- Capsulated
- A part of vaginal flora in 15-20% of humans
- Capsule – made up of N-acetyl neuraminic acid (NANA)
- Tests to identify:
  - Hippurate hydrolysis
  - CAMP test

### CAMP test:

- Demonstrates synergistic β – hemolysis between S. aureus (β – hemolysin) & GBS (Camp factor/β-hemolysin)

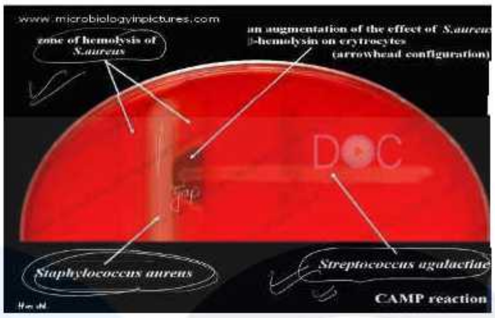

www.microbiologyinpictures.com
zone of hemolysis of S.aureus
an augmentation of the effect of S.aureus β-hemolysin on erytrocytes (arrowhead configuration)
Staphylococcus aureus
Streptococcus agalactiae
CAMP reaction

Fig: 6

- Arrow head configuration of synergistic hemolysis
- Disease: GBS is the most common cause of neonatal meningitis and neonatal septicemia in the West
- DOC: Penicillin

  If allergy: Give Macrolides

Group – D streptococci

| Non enterococcal group | Enterococcal group |
|---|---|
| Genus: Streptococcus | Genus: Enterococcus |
| Mostly sensitive to drugs | (Now in a separate family) |
| Species: S. bovis & S. equinus | Drug resistance is common |

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### S. bovis:

- Diseases:
  - Endocarditis
  - Colonic polyps/cancers
- If isolated from blood, colonoscopy should be done to rule out colonic polyps/cancer
- New name: S. galolyticus

## Enterococcus 00:28:31

- Oval cocci arranged in pairs at an angle to each other (Spectacle-arrangement), singly or short chains

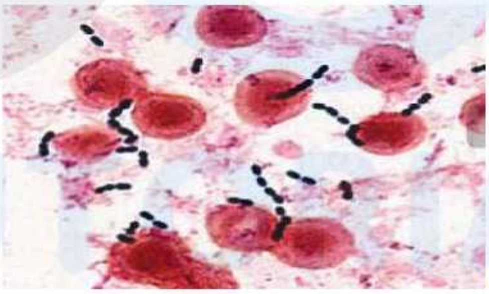


Fig: 7

- They are gut commensals
- Mostly strains are non-hemolytic
- Some strains are β or α hemolytic
- Optimum temperature for growth: 10-15°c
- Optimum pH for growth: 9.6
- Can tolerate 60° for 30 minutes
- Can grow in 40% bile
- Can tolerate 6.5% NaCl

- They are lactose fermenters – pink colonies on MacConkey agar
- PYRase – positive (Pyrrolidonyl aryl amidase )
- Break down esculin to form esculetin
- From black colonies on Bile esculin agar
- Species: MC clinical isolate is Enterococcus faecalis
- Species most resistant to antibiotics is Enterococcus faecium

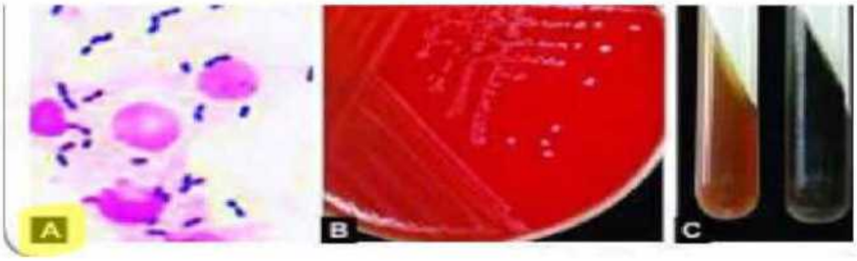
## Diseases:

- UTI – Specially in the setting of pelvic or abdominal surgery
- Septicemia
- Endocarditis
- Meningitis (Rarely)

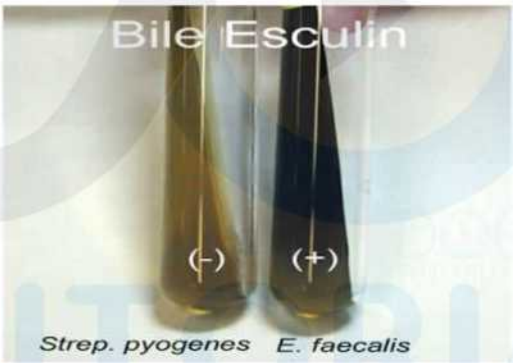
## Treatment:

- Never give Penicillin alone for enterococcal infections except for UTI
- DOC: Penicillin + Aminoglycoside (Synergistic combination)

  Penicillin breaks down cell wall following which Aminoglycoside enters the cell & acts
- If resistant to above, give vancomycin



Enterococcus: A. Gram-positive oval cocci in pairs; B. translucent non-hemolytic colonies on_ blood agar c. Bile esculin hydrolysis test (left- negative, right-positive result, black color due to esculin hydrolysis)


Bile Esculin
(-)
(+)
Strep. pyogenes
E. faecalis

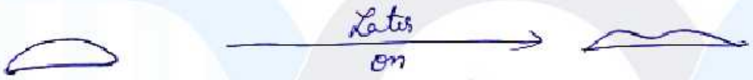
Bile esculin agar

Fig: 8

# STREPTOCOCCUS PNEUMONIAE

## Streptococcus pneumoniae 00:00:26

- Also known as Pneumococcus
- It is α- Hemolytic
- Gram positive, Lanceolate/flamed shaped cocci in pairs (diplococci) and capsulated
- Colonies:



Initially – Dome / convex shaped

Draughtmann appearance / carrom coin appearance (∵ of Autolytic amidase)

- Serotyping: Based on capsular antigens
- Virulence factors:
  - i. Pneumolysin
  - ii. IgA protease
  - iii. Capsule – Antiphagocytic
  - iv. Suicidal enzyme/autolytic amidase

| Viridans Streptococci | Pneumococci |
|---|---|
| • Gram stain: - cocci in chains<br>- No capsule | • Lanceolate-shaped cocci<br>- Capsulated |
| • Colonies: dome-shaped | • Colonies: Draughtsman appearance/Carrom coin appearance |
| • Less pathogenic | • More pathogenic |
| • Inulin fermentation – negative | • Inulin fermentation – positive |
| • Optochin susceptibility: resistant | • Optochin susceptibility: susceptible |
| • Bile solubility test: negative | • Bile solubility test: positive |

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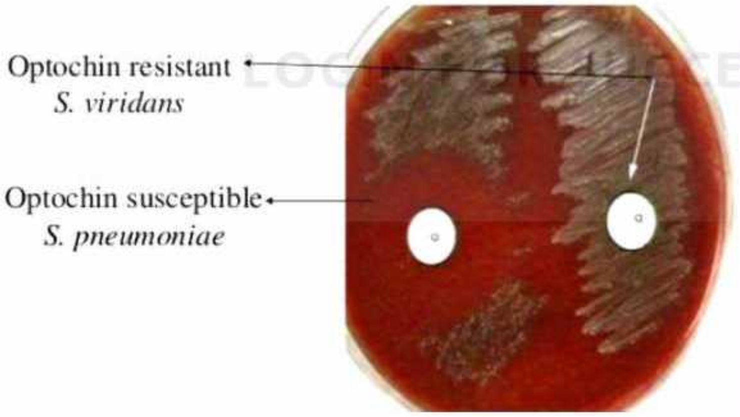

Optochin resistant
*S. viridans*
Optochin susceptible
*S. pneumoniae*

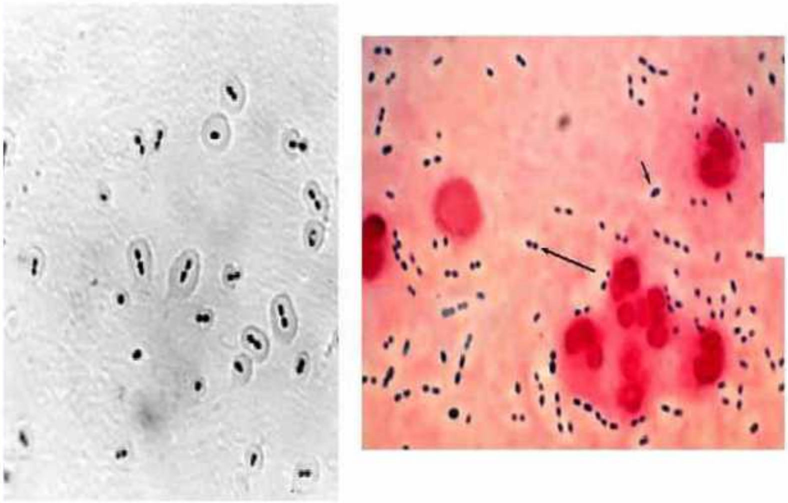
Optochin Susceptibility Test Fig: 1

## Diseases by Pneumococcus:

i. Most common cause of community acquired pneumonia
ii. Most common cause of lobar pneumonia
iii. Most common cause of acute meningitis in adults
iv. Most common cause of acute otitis media in children
v. Most common cause of acute epiglottitis
vi. Most common cause of sepsis in splenectomised people
vii. Important cause of Bacterial sinusitis
viii. Important cause of acute exacerbation of chronic bronchitis

## Treatment:

- DOC: Penicillin
- Resistance can occur due to altered penicillin binding proteins (PBPs)
- If resistant: – Sulfonamides
  Aminoglycosides
  Fluroquinolones
  If resistant → VANCOMYCIN



Streptococcus pneumoniae Fig: 2

# BORDETELLA PERTUSSIS

## Bordetella pertussis 00:00:07

- Causative agent of whooping cough/pertussis
- Also known as "100-day cough" it is a childhood disease
- Gram negative coccobacillus (0.5μm)
- Non-Motile
- Capsulated

### Virulence factors:

1. Pertussis toxin (PT): An exotoxin made of 2 subunits – A&B – "B" subunit attaches to the host cell (Respiratory epithelial cells)

↓

Then A subunit enters

↓

It causes ADP ribosylation of Gi protein

↓

This leads to activation of adenylate cyclase enzyme

↓

ATP → ↑ Cyclic AMP → Leads to edema

2. Fimbrial antigen
3. Adenylate cyclase
4. Tracheal cytotoxin
    - Mode of infection: Inhalation of respiratory droplets – Produced during coughing, sneezing or even talking; rarely by contact

**B. bronchiseptica: Motile, causes "Kennel cough" in dogs**

## Pertussis 00:05:34

- IP: 7-10 days
- Disease characterized by:

1. Catarrhal stage – For 1-2 weeks
   - Fever, headache, Coryza
   - Most infectious stage
   - Smear and culture are positive
2. Paroxysmal stage: Attacks of violent cough
   - Smear and culture may become negative
3. Convalescent stage:

- Frequency of cough decreases
- Ab start to appear

### Complications:

- Hernias
- Sub-conjunctival hemorrhage
- Pneumothorax
- Convulsions

### Diagnosis:

- Sample: Nasopharyngeal aspirate (best)
- Alginate swabs are best for culture
- Dacron swabs – Better for PCR

1. Culture – Gold standard method
   - It is fastidious organism: Grows on
   - Regan lowe medium
   - Bordet-gengou agar

   Colony appearance:

- Mercury drop appearance
- Bisected pearls appearance
- Aluminum paint appearance

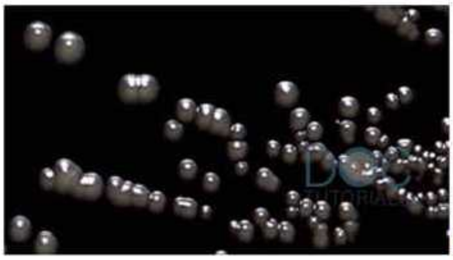


Fig: 1

- If a smear is made from colonies & Gram stain is done, small ovoid coccobacilli are seen, arranged in loose clumps, with clear space in between giving a **thumb print appearance** (figure 2)

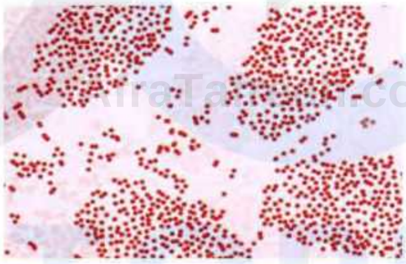


Fig: 2

2.Ab – Detection by ELISA

3.PCR for detecting gene of organism

## Treatment:

- DOC: Macrolides (Erythromycin)
- If resistance – Cotrimoxazole is given.

# PSEUDOMONAS AERUGINOSA

## Pseudomonas aeruginosa 00:00:07

- Strict aerobe

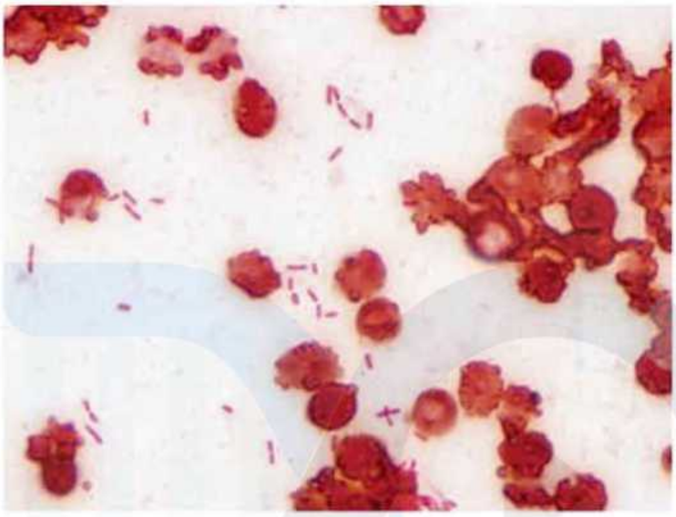


Fig: 1

Gram stain of Pseudomonas aeruginosa

- Gram negative rod
- Motile by unipolar/monotrichous flagellum
- Oxidase positive
- Catalase positive
- Non-lactose fermenter (NLF) – colorless colonies on MacConkey Agar
- Non fastidious
- Pigments – Pyocyanin (Blue)
  - Pyoverdin/Fluorescein (green)

  "Blue pus" – In suppurative infections caused by this organism
- Pigments – are diffusible (Figure 2)

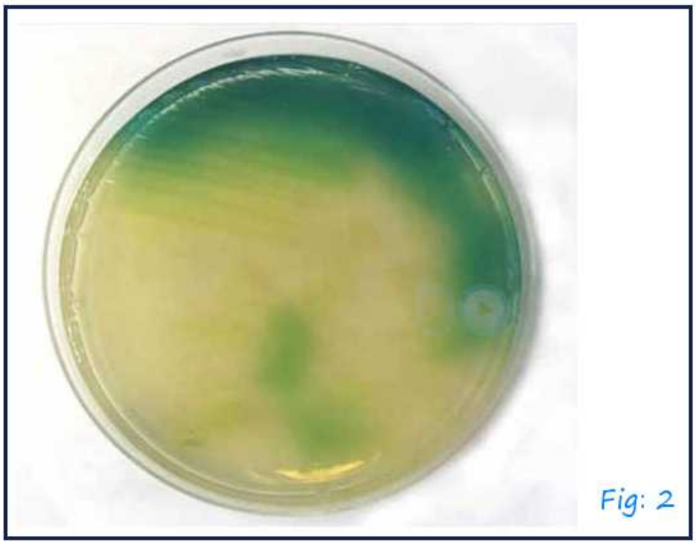


Fig: 2

Diffusible pigments– Entire culture media takes up the color

- Other less important pigments are – Pyorubin (red)
  – Pyomelanin (black)
- Pyocyanin – damages the cilia of respiratory tract
- Selective media for Pseudomonas aeruginosa – **Cetrimide Agar**
- **King's medium**: used for enhancement of pigment production
- Colonies – fruity/grape-like odor because of production of ketones
- **Virulence factors**- 1. LPS/Endotoxin
  2. Enzymes – e.g. proteases
  3. Pyocyanin
  4. Exotoxin- A → most important VF
  MOA is same as the exotoxin of C. diphtheriae
  (Inhibits protein synthesis)

AfraTafreen.com

## Diseases by this organism:

1. Most common cause of keratitis in contact lens wearers
2. Most common cause of burn wound infections
3. Causes malignant otitis externa – seen in diabetics
4. Causes UTI – specially in the presence of urinary catheter
5. Sepsis
6. Diabetic osteomyelitis
7. Hot tub folliculitis
8. Swimmer's ear (milder otitis externa)
9. Ecthyma gangrenosum: Black necrotic skin lesions (in immune compromised people like those with neutropenia, HIV positive people)
10. Shanghai fever
11. Endocarditis (In Intravenous drug abusers) – right sided endocarditis usually
12. Pneumonia – i. Nosocomial pneumonia
    ii. Pneumonia in cystic fibrosis patients – by slime producing stains

Burn wound Infection

Endocarditis

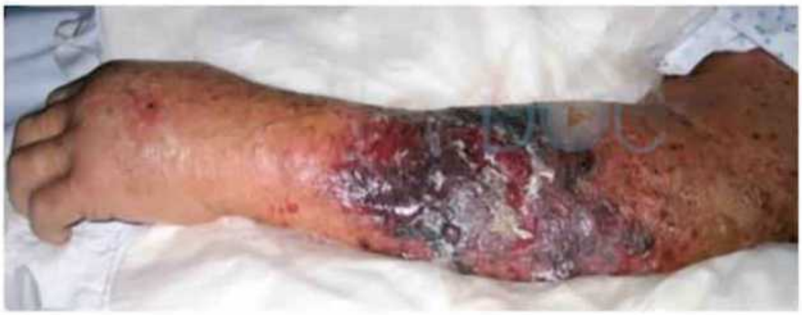
Pneumonia

Sepsis

External malignant otitis

UTI

D
O } Diabetic osteomyelitis



Ecthyma gangrensum

Fig: 3

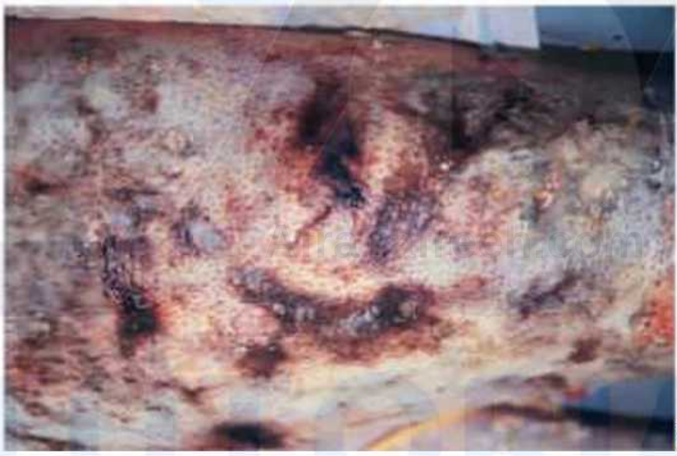


Fig: 4

Pseudomonas infection of burn wound

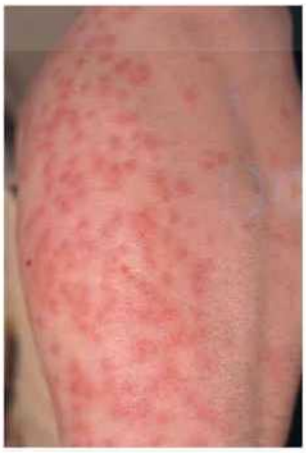


Fig: 5

Pseudomonas folliculitis

### Treatment:

- Pseudomonas species are inherently resistant to most of the antibiotics. Only limited antimicrobial agents have antipseudomonal action, such as:
  - –Pencillins: Piperacillin, mezlocillin, ticarcillin
  - –Cephalosporins: Ceftazidime, cefoperazone, ceftolozane and cefepime
  - –β – lactam/ β -lactamase inhibitor combination (piperacillin-tazobactam and cefoperazone-sulbactam)
  - –Carbapenems: Imipenem, meropenem and doripenem
  - –Monobactum: Aztreonam
  - –Aminoglycosides: Tobramycin, gentamicin, amikacin
  - –Quinolones: Ciprofloxacin, levofloxacin
  - –Polymyxins: polymyxin B, colistin.

## Legionella pneumophila 00:17:34

- Strict aerobe
- Gram negative rod
- Motile organism
- Fastidious organism

Selective medium is **Buffered Charcoal Yeast Extract (BCYE) agar**

↓

Has iron and cystine in it

- Organism is water loving, it is present in
  i. Water bodies – lakes, streams
  ii. Water of air conditioning & cooling plants
  iii. Lives inside Acanthamoeba
- Most common mode of infection – Aspiration
- Other modes – Inhalation
  - Direct inoculation into lungs
- Risk factors: Smokers
  Alcoholics
  Transplant recipients

Diseases:

- In Immunocompetent people – causes **PONTIAC FEVER**
  –Self-limiting, influenza-like illness, no pneumonia.
- In Immunocompromised individuals – causes **legionnaire's disease**
  –It is characterized by Pneumonia
  Hyponatremia
  Diarrhea
  –Relative bradycardia is present (Inconsistent finding)

- Most common extrapulmonary complication is Carditis

## Diagnosis:

Sample – 1. Bronchoalveolar lavage (BAL)
2. Sputum

i. Gram stain: Shows lot of neutrophils/pus cells without organisms

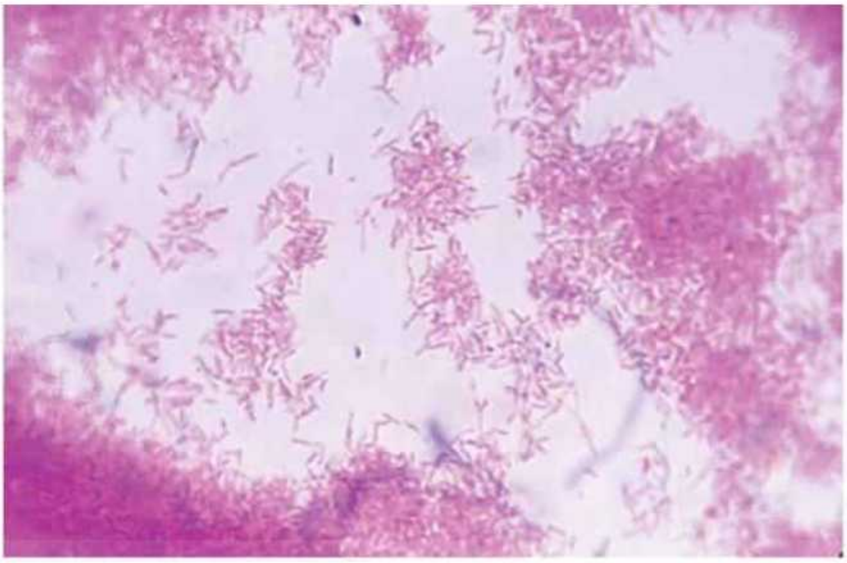
**L.pneumophilia: seen on Gram stain on prolonged duration of counterstain.**

ii. Direct fluorescent antibody (DFA) stain – best stain
iii. Silver stain
iv. Culture on BCYE agar
v. Urinary antigen detection for Serotype – 1
↓
Most common serotype causing human infection & disease

- Note: Pneumonia by this bacterium is atypical because:
  - It is an interstitial pneumonia
  - Organism does not grow on routine culture media

## Treatment:

Macrolides or Quinolones

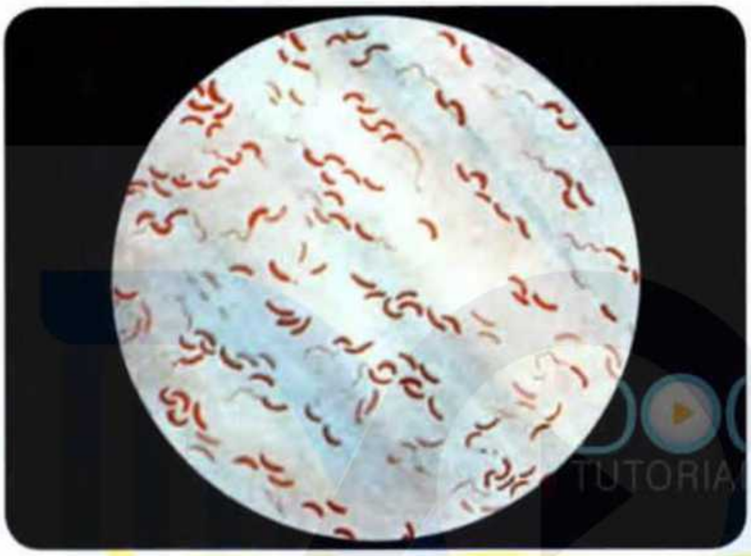


Gram stain of L. pneumophila Fig: 6

# VIBRO CHOLERAE & HALOPHILIC VIBRIOS

## Vibrio cholerae 00:00:11

- Gram negative, comma shaped/C-shaped/curved rods



Gram stain of Vibrio cholerae Fig: 1

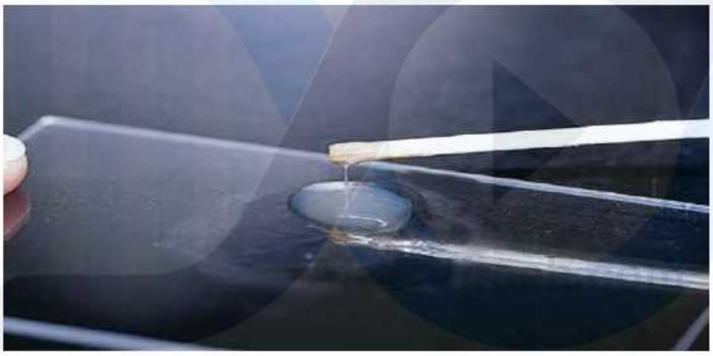
- Under-microscope: Fish in stream appearance
- Catalase positive
- Oxidase positive
- Facultative anaerobes
- Need alkaline pH to grow (pH: 8.2 – 8.5)
- Motile – Unipolar/Monotrichous flagellum
- Motility: Shooting star/darting motility
- Shows **positive cholera red reaction** – combination of

  - Indole test: positive
  - Nitrate reduction test: positive

- COIN positive (Catalase, Oxidase, Indole, Nitrate reduction)
- String test positive:

  On a slide, suspension of V. cholerae with 0.5% sodium deoxycholate is taken & mixed

  - Suspension loses it turbidity & becomes mucoid
  - A string forms when the mixing stick is withdrawn away from the slide



String test Fig: 2

- String test – used to differentiate V. cholerae (positive) from Aeromonas species and P. shigelloides (negative)
- V. cholerae is:
  - NLF
  - Sucrose fermenter
  - Non-halophilic

## Disease:

- Cholera is an acute diarrheal illness
- Mode of infection: Faeco-oral route
- Infective dose: $10^5 - 10^8$ organisms (same as Salmonella typhi)

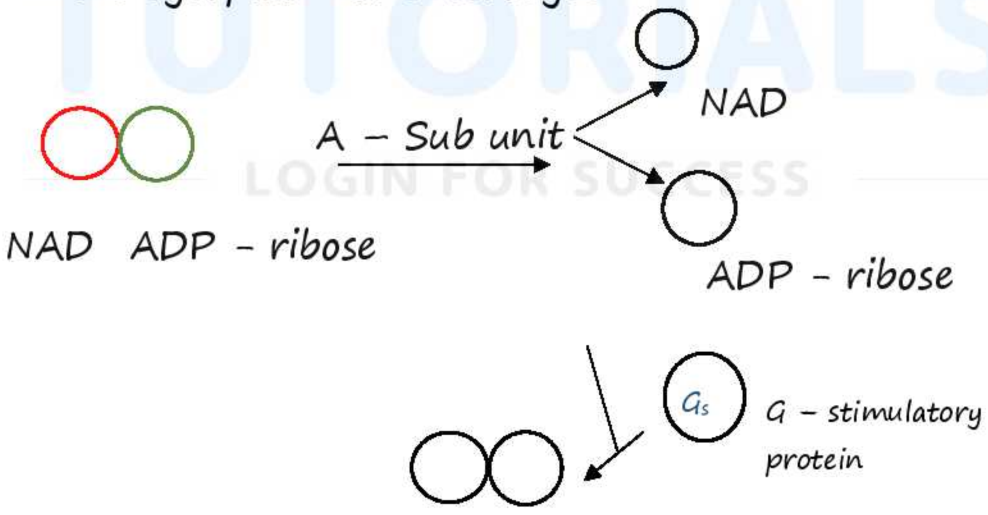
  ∵ this organism is acid-labile
- I.P: 16-18 hours
- Most important virulence factor: Cholera-toxin/Choleragen

  Cholera toxin is an exotoxin, made up of A&B subunits

- A&B subunits are bound to each other
- B subunit binds to GM1 ganglioside receptor of enterocyte, then

  A subunit enters the enterocyte.

  In the cytoplasm of enterocyte:


NAD
ADP - ribose
A - Sub unit
NAD
ADP - ribose
Gs
G - stimulatory protein

ADP - ribosylation of $G_s$ protein

↓

Stimulates Adenylate cyclase

- Adenylate cyclase converts:

$$ATP \xrightarrow{\text{Adenylate cyclase}} \uparrow cAMP \text{ (abundant)}$$

↓

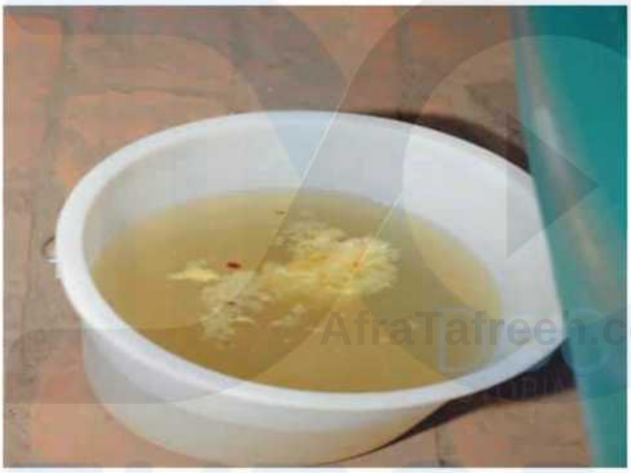
– Increased $Na^+$ secretion

– Decreased absorption of $HCO_3^-$

↓

Secretory Diarrhea

- No Blood or pus cells present in faeces
- Stools are called as "Rice watery Stools"



Rice watery stools Fig: 3

- Cholera toxin: is 100-1000 times more potent than labile toxin of ETEC.

## Mainstay of treatment of cholera:

- Fluid & Electrolyte replacement

Cholera toxin – Gene for cholera toxin present on a phage (phage – encoded)

- **TCP/Toxin coregulated pilus**: Type of Type – IV fimbriae
  - Helps in attachment to enterocytes
- **Zona occludens toxin**: Disrupts the tight junctions between mucosal cells.
- Siderophore

> - LPS of V. cholerae – no role in pathogenesis of cholera

> - TCP named so because gene for cholera toxin, pilus and some other VFs are together coregulated by – TOXR gene.

- V. cholerae – classified based on somatic 'O' Ag:

  ↓

  $O_1$ to $O_{139}$ serotypes
- $O_1$ – has 2 biotypes

  i. Classical

  ii. Eltor

- $O_1$ – main cause of cholera
- So far: 7 pandemics of cholera

1st – 6th ↓ Caused by classical biotype

7th ↓ Caused by Eltor biotype

| | Classical | Eltor |
|---|---|---|
| 1. Severity of the disease | More severe | Milder disease |
| 2. Secondary attack rate | Higher | Lower |
| 3. Carrier state | Less common | More common |
| 4. Hemolysis of sheep RBC | Absent | Present |
| 5. Haemgglutination of chicken RBCs | Absent | Present |
| 6. Voges proskauer test | Negative | Positive |
| 7. Polymyxin – 'B' sensitivity | Susceptible | Resistant |
| 8. Susceptibility to Group-IV phage | Susceptible | Resistant |
| 9. Susceptibility to Group-V phage | Resistant | Susceptible |

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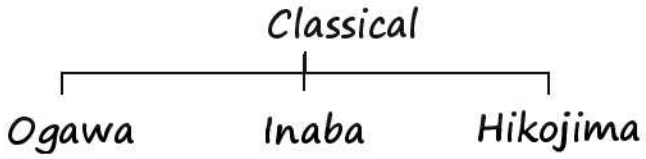
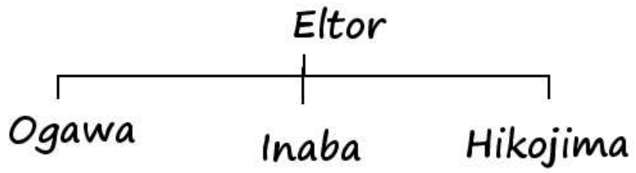
- Biotypes are further divided into serotypes:





- Currently, most common serotype of V.cholerae:

  $O_1$ Eltor ogawa

- $O_{139}$ serotype:
  - Caused outbreak of cholera in Chennai in 1992 – 1993
  - 1st isolated in Chennai
  - Called as "Bengal strain" because it spread along the Bay of Bengal
  - Capsulated strain
  - Slightly invasive

## Diagnosis of cholera:

- Specimens: 1. Best – mucous flakes

  2. Faeces

## Transport media:

i. Venkatraman Ramakrishnan (VR) medium for
  - V. cholerae

ii. Cary-Blair medium
  - Universal transport medium

I. Enrichment broth: Selenite 'F' broth

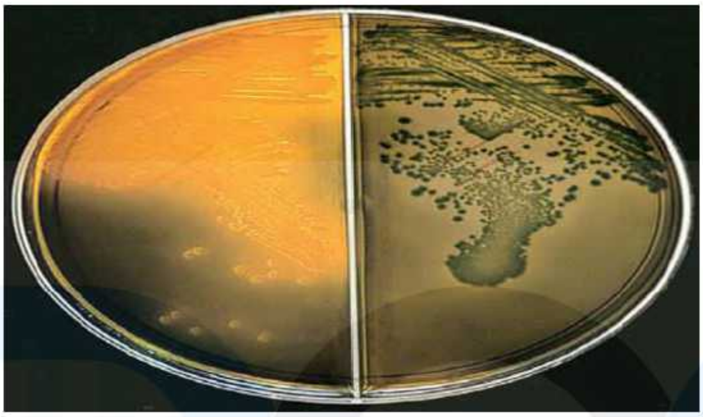
Alkaline peptone water

- Faeces are incubated in enrichment broth for 4-6 hours, then subcultured on selective media
- Selective media used are: 1. MacConkey agar-NLF colonies

2. TCBS agar-yellow colonies

(Thiosulfate citrate bile salt sucrose agar)

(Yellow colonies because V. cholerae ferments sucrose)



- TCBS agar: Left – V. cholerae; right – V. parahaemolyticus

Fig: 4

## Halophilic Vibrios 00:38:12

- They tolerate NaCl concentration > 6%

- V. cholerae: Optimum NaCl concentration 0.5-1% & can tolerate upto 6%

- Present in marine environment/marine waters
- Halophilic vibrios are:

i. V. parahaemolyticus
  - Capsulated organism
  - Shows bipolar staining/safety pin appearance
  - Motile by peritrichous flagella

- Mode of infection: By intake of sea food
- Disease: prevalent in Japan
  Gastroenteritis – watery diarrhea, sometimes dysentery

- **No enterotoxin yet discovered**
- Shows KANAGAWA PHENOMENON on Wagatsuma agar

  Wagatsuma agar: - Is a blood agar with high salt content

  - Human blood is used

  Kanagawa phenomenon: Strains which cause β hemolysis on this agar are pathogenic to humans, non-hemolytic strains are non-pathogenic

ii. V. vulnificus – Latin word → Means wound maker

- Present in sea
- Mode of infection: infection of wounds by contact with sea water
- Causes septicemia in people with chronic liver disease and iron overload
- It is the only species in genus vibrio, which is LF

iii. V. alginolyticus

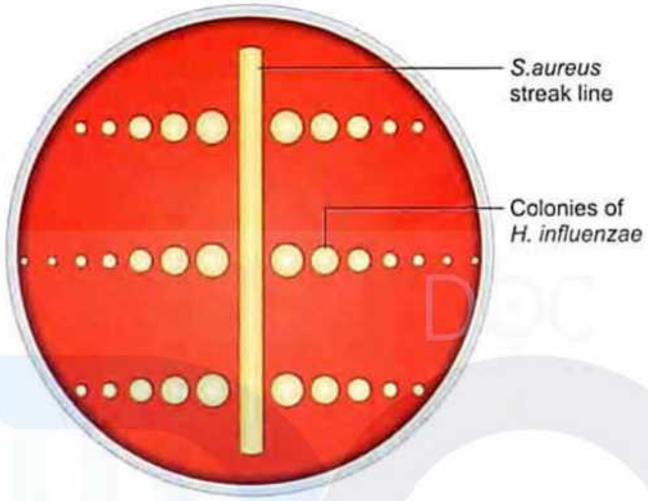
- Causes infection of eyes, ear, wounds
- Most salt-tolerant species of Vibrio – can grow at NaCl concentration > 10%

# HAEMOPHILUS INFLUENZAE

## Haemophilus influenzae 00:00:12

- Does not cause influenza
- Does not grow on blood agar
- It is also know as "**Peiffer's bacillus**"
- Facultative anaerobe
- Gram negative coccobacillus
- Oxidase positive
- Catalase positive
- Non motile
- Capsule – can be present/absent
- Both capsulated & non capsulated strains can cause disease
- Fastidious organism
- It needs – 2 factors for growth
  - X-factor – Is heat stable hemin
  - V-factor – Is NAD (Nicotinamide adenine dinucleotide)
- **On blood agar:** Growth is scanty; because only **X-factor** is available & **V-factor** is largely intracellular
- **Sheep blood agar:** has NADase → which destroys V-factor
- **On chocolate agar:** Both 'X' & 'V' factors are present
- **Satellitism** → Phenomenon which shows requirement of 'V' factor for the growth of H. influenzae

- Maximum size of H. influenzae colonies are seen near the S. aureus streak since V-factor is produced by S. aureus


S.aureus streak line
Colonies of H. influenzae

Satellitism of H. influenzae

Fig: 1

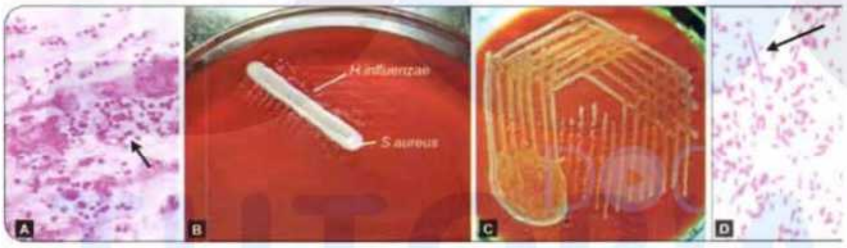

A
B
H.influenzae
S.aureus
C
D

Fig: 2

A. Gram-stained smear showing pleomorphic gram-negative bacilli; B. Satellitism of H. influenzae around S. aueus streak line; C. Colonies of H. influenzae on chocolate agar; D. Colony smear showing pleomorphic gram-negative bacilli

## Capsule:

- Based on antigens of polysaccharide capsule, there are 6-serotypes of H. influenzae

**Non capsulated strains are – Non typeable**

- Capsulated strains – 6 serotypes
  - a, b, c, d, e, f
- Serotype-b:
  - Causes most severe disease in humans
  - Most common cause of human infection
  - Its capsule is made up of Polyribosyl Ribitol Phosphate (PRP) capsule

## Diseases caused by H. influenzae 00:00:12

### Diseases-caused by H. influenzae

| **Invasive:** | **Non-invasive:** |
|---|---|
| Blood stream in invaded | Spread occurs by contiguous mucosal spread |
| By capsulated strains | By non-capsulated strains |
| **Example:** | |
| 1. Epiglottitis:<br>• Now the MCC of epiglottitis is Streptococcus pneumoniae | 1. Community Acquired Pneumonia (CAP)<br>• 2nd MCC of CAP |
| 2. Meningitis – seen in children 2months – 6 years of age<br>• Occurs due to the absence of anti-PRP Abs<br>• Complication of this meningitis is subdural effusion | 2. Lobar pneumonia: 2nd most common cause |
| 3. Cellulitis in infants | 3. Otitis media: 2nd most common cause in children |
| 4. Pneumonia in infants | 4. Acute exacerbation of chronic bronchitis – Most common cause |

## Causes of community acquired pneumonia:

- Most common cause: Pneumococcus
- 2nd most common cause: H. influenzae (Non capsulated strains)
- 3rd most common cause: Chlamydophila pneumoniae

## Treatment:

- DOC:
  - Ceftriaxone
  - Cefotaxime
- Penicillin is not used because 20-35% isolates are β-lactamase producers.

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# HAEMOPHILUS AEGYPTIUS & H. DUCREYI

## Haemophilus aegyptius/ Koch's week bacillus

00:00:11

- Needs both X-factor & V-factor
- 2- important diseases:

**Important diseases**

**Pink eye**
- Highly contagious conjunctivitis

**Brazilian purpuric fever**
- Fever, purpura, hypotension shock

## H. ducreyi

00:01:17

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- Needs only X-factor to grow, not V-factor
- Causes STI: Chancroid/Soft sore
- Chancroid:
  - Painful genital ulcer which bleeds easily
  - Surrounding inflammation is absent
  - Associated painful inguinal lymphadenopathy (Buboes)
- H. ducreyi – Important cause of genital ulcer disease in developing countries
- H. ducreyi infection – Increases the chance of acquiring HIV infection and increases chances of transmitting HIV to others
- IP: 4-7 days

## Diagnosis:

- Make a smear from pus from ulcer/ swab from edge of ulcer/ L.N aspirate:

  1.Stain the smear with Giemsa

  - Tram track appearance/ Rail road track appearance

  2.Culture:

  On chocolate agar supplemented with 1% isovitalex

  - Made selective by adding vancomycin
  - 5-10% Co$_2$ should be provided
  - High humidity should be present
  - Temperature of 35°c should be there
  - Incubate for 2-3 days
- Small grey, translucent colonies (1-2mm diameter) appear

## Treatment:

- Single oral dose of 1gm Azithromycin is the DOC

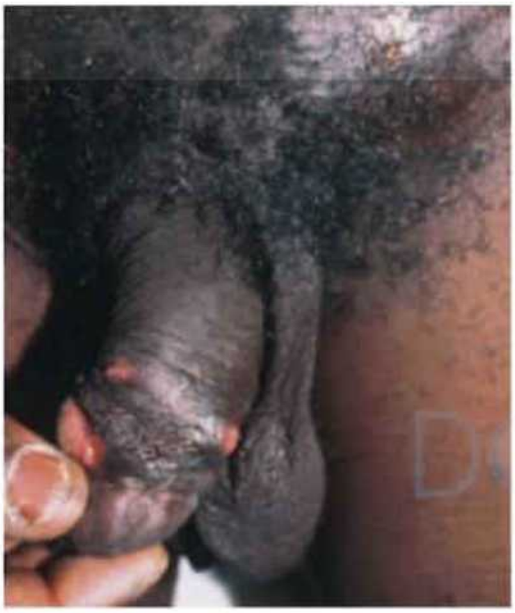


Fig: 1

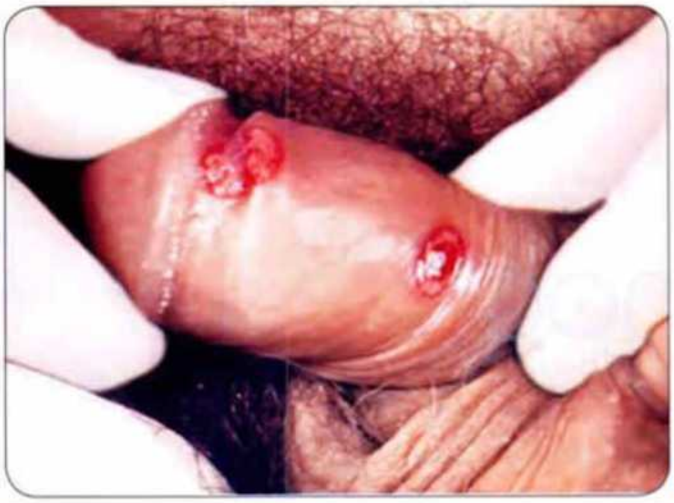
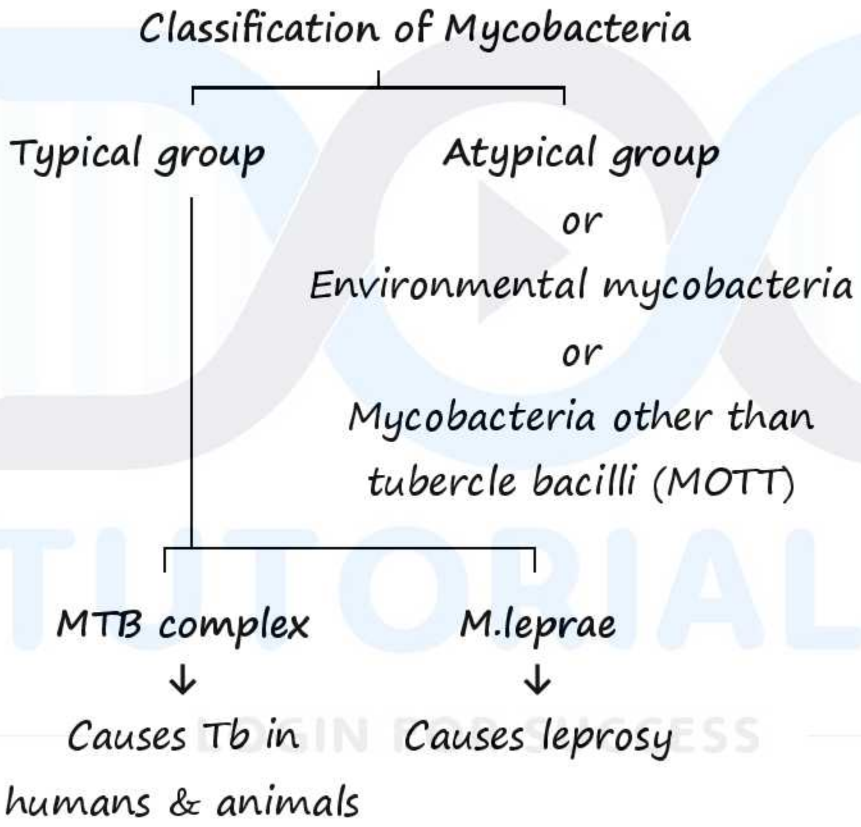


Fig: 2

# MYCOBACTERIUM TUBERCULOSIS PART-I

## Mycobacteria 00:00:10

- They are acid fast
- They are weakly gram positive
- Acid fast because of lipid – mycolic acid in their cell wall



Classification of Mycobacteria

Typical group

Atypical group
or
Environmental mycobacteria
or
Mycobacteria other than tubercle bacilli (MOTT)

MTB complex → Causes Tb in humans & animals

M.leprae → Causes leprosy

DOC TUTORIALS
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**MTB complex:**

- M.tuberculosis (most common cause of Tb in humans)
- M. bovis
- M. africanum
- M. microti

## Mycobacterium tuberculosis 00:05:17

- Most common causes of Tb in humans
- It is an obligate aerobe
- It is facultative intracellular & induces granuloma formation in host tissues
- Most common mode of infection – Inhalation of droplet nuclei (<5µm size)
- Most common type of Tb in humans – pulmonary Tb

## Types of pulmonary Tb 00:06:46

Types of pulmonary Tb

| Primary Tb | Secondary/post primary |
|---|---|
| Occurs after first infection by the microorganism | i. Occurs because of reactivation of dormant bacilli therefore, also known as reactivation Tb<br>– Reactivation occurs in compromised-immunity cases<br>ii. Reinfection |

**Primary pulmonary Tb:**

- Lung lesion: found in upper segment of lower lobe & lower segment of middle lobe
- This lung lesion is known as GHON's focus
- Ghon's focus + associated hilar lymphadenopathy is known as Ghon's complex
- In India: 1/3rd of population is infected with Tb

- If the immunity decreases in this infected population – reactivation of dormant bacilli can occur

↓

Such people can manifest clinical features and become infectious to others

- 80% of Tb cases – pulmonary Tb
- Rest 20% of Tb cases – Extrapulmonary Tb cases (EPTB)

## Extra pulmonary Tb-cases 00:11:12

- Occurs because of hematogenous dissemination of organisms in various organs

### Types of extra pulmonary Tb:

i. Most common type of EPTB is tuberculosis lymphadenitis (35% of all cases)
  - Lymph nodes involved are
    - Posterior cervical
    - Supraclavicular
  - Lymph node Tb presents as – painless swelling in the neck region without any warmth or color change

ii. Pleural Tb: 20% of all EPTB cases
  - Manifests as pleural effusion
  - For diagnosis, pleural biopsy is collected

iii. Tb of upper airways
  - Larynx

  - Epiglottis
  - Pharynx

iv. Genitourinary Tb

  a. Renal Tb

  b. In males – epididymis is most commonly involved

  c. In females – Fallopian tubes & endometrium are involved

- Tb – is a common cause of sterility in females in INDIA

v. Skeletal Tb

- Involves weight bearing joints
  - Spine: Pott's spine (or) tuberculous spondylitis
  - Hips
  - Knees

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vi. Tb of CNS: In children

- Manifests as meningitis (aseptic) & tuberculoma
- Aseptic meningitis caused by following bacteria

  a. M. tuberculosis

  b. Treponema pallidum

  c. Leptospira interrogans

vii. GIT Tb – in caecum & terminal ileum

- GIT Tb can occur because of:
  - Swallowing of sputum
  - Intake of unpasteurized milk
  - Hematogenous spread

viii. Tb-pericarditis

- Seen in areas with low Tb prevalence

- Usually seen in elderly people
- It occurs as direct extension from adjacent lymph nodes or following hematogenous spread

ix. Skin lesions

- Scrofuloderma:
    - It is the tubercular involvement of skin because of direct extension usually form underlying lymphadenitis
- Lupus vulgaris: Apple jelly nodules on face in females

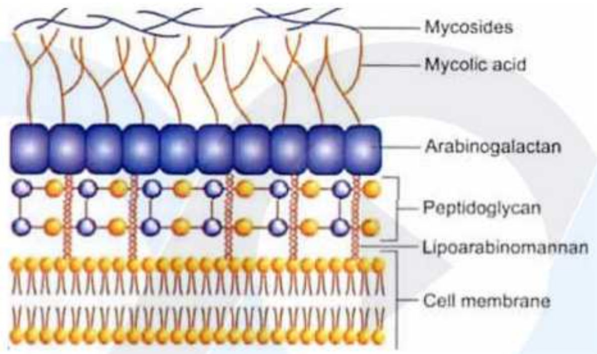
x. Miliary Tb/disseminated Tb

- Occurs because of hematogenous spread of Tb bacilli
- This leads to the formation of 1-2mm granulomatous lesions resembling millet seeds in various organs
- Usually seen in HIV infected individuals.

# MYCOBACTERIUM TUBERCULOSIS PART - II

## Antigens of Mycobacterium tuberculosis 00:00:11

- Cell wall Ags or insoluble Ags
- Cytoplasmic (or) soluble Ags
  - Include Ags – 5,6,60
  - These Ags can be used for serodiagnosis


Mycosides
Mycolic acid
Arabinogalactan
Peptidoglycan
Lipoarabinomannan
Cell membrane

Cell wall of M. tb Fig: 1

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Cell wall antigens:

### Mycolic acid

- Responsible for:
  - Acid fastness
  - Decreased penetration of antibiotics & stains

### Arabinogalactan layer

- It facilitates survival of M. tb within macrophages

### Lipoarabinomannan (LAM)

- Important Ag & helps in attachment of organism to macrophages
- It is also a target Ag used in diagnosis of Tb

## Lab diagnosis of Tb 00:03:32

### For pulmonary Tb

- Specimen:

**2 sputum samples**

**Spot specimen**
- Taken under supervision
- On the same day

**2nd sample**
- Next day (early morning)

- Or 2 spot samples are taken atleast 1hour apart on the same day
- Quantity of sample: 2-5ml (preferably mucopurulent part)
  - Without blood tinge

### For extra pulmonary Tb

- Samples are collected depending on the site involved

| Extrapulmonary specimens | |
|---|---|
| Sterile site specimens collected aseptically | |
| Optimum specimens | CSF, spinal, pericardial, synovial, ascitic, blood and bone marrow, pleural biopsy, tissues (collected in sterile saline) |
| Suboptimal specimens (organism load is less) | Pleural fluid (20-50 ml is collected and centrifuged)<br>Blood (indicated only for disseminated TB and co-infected with HIV) |
| Specimens containing normal flora | |
| Swabs | Considered suboptimal specimens<br>The only recommended swabs are:<br>- Laryngeal swabs: Collected early morning in empty stomach (or)<br>- Swab form discharging sinus |
| Urine | Three early morning specimens collected (500mL/specimen, centrifuged) on different days as TB bacilli in urine are shed intermittently |
| Stool | For disseminated TB in HIV infected patients and infants |
| Other respiratory specimens | Bronchial secretions (2-5mL)<br>Bronchoalveolar lavage (20-50mL)<br>Transbronchial and other biopsies |
| Gastric lavage | Recommended for children (tend to swallow sputum), or ICU patients (aspiration)<br>Early morning lavage should be collected and processed early (<4hours) |

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- Note: Samples for cultures should never be collected in formalin.
  - If histopathological examination is required, two samples should be collected

- For sputum sample & for extra-pulmonary specimens taken from non-sterile sites:
  - Digestion
  - Decontamination
  - Concentration
- Digestion: is to liquify pus cells & to homogenize the sample of the specimen
- Decontamination: is to inhibit the normal flora
- Concentration: To increase the yield
- Digestion, decontamination & concentration are not needed for
  - Molecular method of diagnosis (e.g., CBNAAT)
  - Samples taken from sterile sites (or) aseptically taken samples

## Concentration methods 00:10:36

i. Modified petroff's method

- 4% NaOH is used
- Sputum sample is thoroughly mixed with equal volumes of 4% NaOH & centrifuged

- The sediment is neutralized by treating it with phosphate buffer saline at a pH of 6.8
- This method is recommended for LJ culture

ii. NALC/N-acetyl L-cysteine + 2% NaOH

- This method is superior to modified petroff's method
- NALC – liquifies the sputum sample
- Also, NALC kills the normal flora in sputum sample
- It is recommended for automated culture media

## Methods of diagnosis 00:13:35

### i. Direct microscopy using ZN staining

- Smears are prepared from thick mucopurulent part of sputum or sediment obtained after concentration

### Interpretation

- Negative result: At least 100 OIFs should be scanned for 10-15min
- Positive result: If long, slender, beaded, less uniformly stained red/ pink AFB are seen

### Disadvantages

- One cannot distinguish M.tb from saprophytic mycobacteria present in tap water (or) from commensals present in gastric lavage (or) urine

  - Hence, positive result is only a presumptive diagnosis
- Less sensitive
  - Because at-least ≥10,000 bacilli/ml in specimen are required for positive test
- This method can't determine viability of the organisms

---

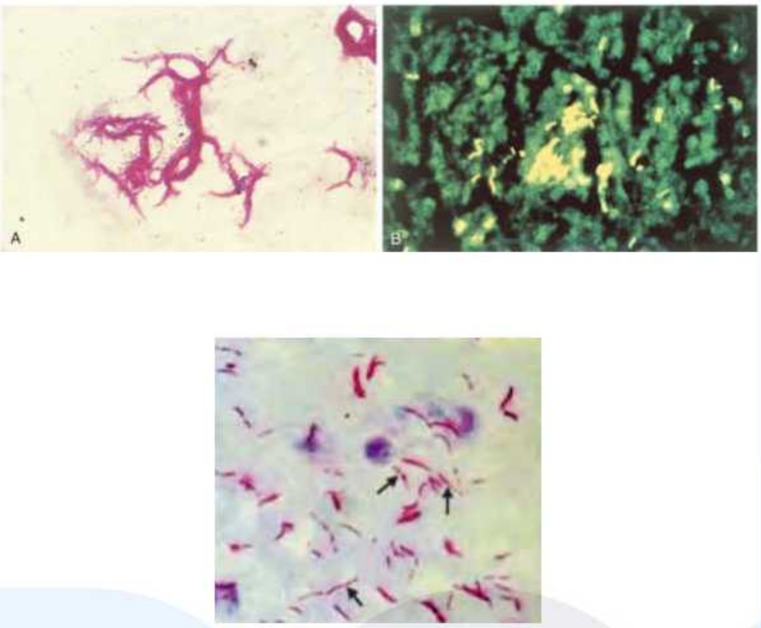
## Kinyoun's cold AF staining 00:17:52

---

- Heating is not needed
- Phenol concentration is increased in carbolfuchsin
- Duration of carbol fuchsin staining is more

### ii. Fluorescence staining - 3 steps

| Duration | Stain | |
|---|---|---|
| 20 min | 0.3% auramine stain | Primary stain |
| 3 min | 0.5% acid alcohol | Decolorizer |
| 1 min | 0.1% potassium permanganate | Counterstain |



ZN staining

Fig: 2

### Advantages of fluorescence staining:

- Rapid – under 20x or 25x objective, 100 fields can be seen within 2 min
- More sensitive than ZN staining

### iii. Culture

- Gold standard for diagnosis of Tb
- LJ medium (Lowenstein Jensen Medium) is used most commonly

### Advantages

- More sensitive: 10-100 bacilli/ml are needed
- Confirms viability
- Drug susceptibility testing can be done
  - LJ medium: Egg based medium

## Components

- Coagulated hen's egg
- Mineral salt solution
- Asparagine
- Malachite green (selective agent)

## Incubation

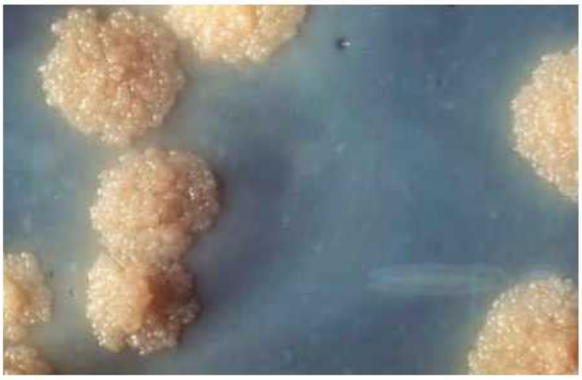
- Done for 6-8weeks because the generation time is 12-13 hours

## Colonies of M.tb

- Rough
- Tough
- Buff

## Colonies of M.bovis

- Smooth
- Moist
- Grey-white
- Break easily when touched



M.tb colonies on LJ medium Fig: 3

## Conventional liquid media

- Kirchner's medium
- Middle brook 7H9 medium

## iv. Automated liquid culture

- Detects the growth of microorganism
- MGIT – Mycobacterium Growth Indicator Tube
- Detects growth of M.tb
- Performs drug susceptibility testing against 1st and 2nd line of anti-Tb drugs
  - Principle of MGIT

Broth + Sample with M.tb → Increase in fluorescence

- Broth has an $O_2$-sensitive fluorescent compound
  - When M.tb is added, the $O_2$ is consumed in the broth
  - Quenching of fluorescence is inhibited & fluorescence is increased

Colonies on LJ medium

(or)

Positively flagged automated broth

↓

AFB staining is done with it

↓

If AFBs are seen – further test are done to identify the species

### v. MPT-64 antigen detection

- Detection by immunochromatographic technique
- MPT-64 Ag is a 28Da Ag
- Very specific for M.tb complex
- Negative for MOTT

### vi. Biochemical reactions

- Nitrate reduction test
- Niacin test
- Pyrazinamidase test

All these are positive for M.tb

### vii. Rabbit pathogenicity test

- M.bovis is pathogenic for rabbit
- M.tb is not pathogenic for rabbit

### viii. Gene xpert – WHO recommended

- CBNAAT – It is cartridge based nucleic acid amplification technique
- It is an automated PCR technique
- Turnaround time – 2hours
- Detects rifampicin resistance
- Preferred method for diagnosis of EPTB

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Fig: 4

## Advantage

- No contamination
- Detection limit of gene xpert is: 131 org/ml
- Sensitivity of gene xpert: 88% sensitive and 99% specific for detecting M.tb
- For detecting rifampicin resistance:
  - Sensitivity: 95%
  - Specificity: 98%

# MYCOBACTERIUM LEPRAE

## Mycobacterium leprae 00:00:10

- Obligate intracellular organism and obligate aerobe
- It cannot be grown/isolated on artificial culture media
- Disease: Leprosy
- The organism involves/infects cooler parts of the body like skin, peripheral nerves, eyes and testes and upper respiratory tract
- Generation time:12-13 days
- Genome of M. leprae: has undergone reductive evolution

## Ridley Jopling's classification 00:04:03

Ridley Jopling's classification

| LL | BL | BB | BT | TT |
|---|---|---|---|---|
| Lepromatous | Borderline lepromatous | Borderline leprosy | Borderline tuberculoid | Tuberculoid |

- In India BT type is most common type
- Lepromatous leprosy: has got bad prognosis. Cell Mediated Immunity (CMI) is decreased
- Tuberculoid leprosy: prognosis is good. Good CMI

## Difference between LL and TT 00:05:48

| TT | LL |
|---|---|
| Paucibacillary | Multibacillary |
| Good CMI | Decrease CMI |
| $T_H1$ response is predominant | $T_H2$ cells – predominant |
| Less infectious | More infectious |
| Lepromin test is positive | Lepromin test – negative |

- Multibacillary cases: are the most important source of infection
- Modes of transmission
  - i. Nasal droplet infection: inhalation of droplet nuclei
    - This is the most common mode of infection
    - A sneeze from a patient of LL:$10^{10}$ bacilli are released
  - ii. Contact transmission: by skin
    - Direct contact: from person to person
    - Indirect contact: contact via infected soil or fomites (Linen, clothes
  - iii. Direct dermal inoculation: during tattooing

### Incubation period: 3-5 years

### Clinical features

Chronic granulomatous disease

- Clinical features of TT:

  - Skin lesions are few and asymmetric and sharply demarcated
  - Skin lesion: macular, depigmented/hypopigmented anesthetic patches with elevated borders
  - Nerve involvement is early
  - Nerves can be enlarged and thickened
  - Nerve abscess can be formed
  - Most common nerves involved are
    - Ulnar nerve
    - Posterior auricular nerve
- Clinical of LL:
  - Skin lesions are many, symmetrical and with irregular margins
  - Skin lesions – nodules (due to Ag-Ab complexes)
  - If nodules are present on face: leonine facies
  - Leonine facies (figure below)

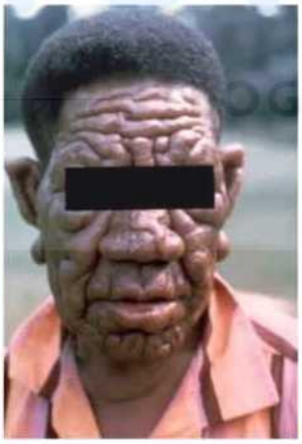

Fig: 2

  - TT leprosy (figure below)

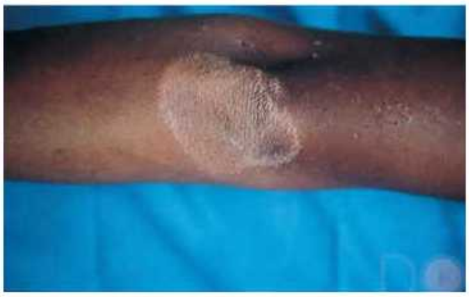

Fig: 3

- LL leprosy (figure below)

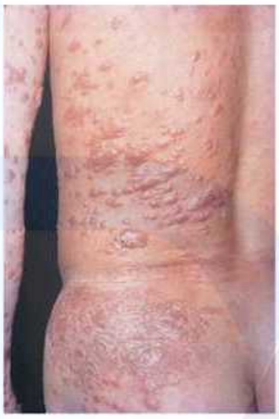

Fig: 4

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- Loss of eyebrows/eyelashes
- Loss of nasal septum leading to saddle nose deformity
- Nerve involvement is late
- These LL patients shed organisms in large numbers in nasal secretions
- LL patients: are most infectious
- If not treated, other organs are involved like:
  - Reticuloendothelial system
  - Eyes
  - Kidney
  - Bones
  - Testes

- In the macrophage, bacteria are arranged in bundles (Cigar bundles / Globi) in a lipid like substance called Glia
- This macrophage with cigar bundles is called as foamy macrophage/lipid laden macrophage
- Purely neuritic leprosy:
  - Only nerves are involved
  - It is a type of tuberculoid leprosy

### Lepra reactions (Hypersensitivity reactions)

| Type – 1 | Type -2 |
|---|---|
| It is a type- IV Hypersensitivity reaction | Type – III Hypersensitivity reaction |
| It is seen in borderline leprosy | It is seen in BL/LL |
| Clinical features: inflammation of previous lesions | Clinical features: Eruption of crops of painful erythematous papules which become nodular |
| If type-1 Lepra reaction occurs before treatment, progression is likely towards LL<br>If it occurs after treatment → progresses towards TT | It is usually seen after starting the treatment |

| $T_H1$ cells | $T_H2$ cells |
|---|---|
| Treatment: Steroids | Treatment:<br>•Steroids<br>•Thalidomide<br>•Clofazimine<br>•Antipyretics |

## Lab diagnosis 00:21:33

1) Smear examination – using modified ZN Staining:

$5\%$ $H_2SO_4$ – decolorizer

- To see AFB in the smear
- 6 samples taken:
  - 4 samples from skin
    - Forehead
    - Cheek
    - Chin
    - Buttock
  - 1 sample from earlobe
  - 1 sample from nasal mucosa by nasal blow/nasal scraping
- Slit skin smear technique to collect samples from skin and earlobes
- Edge of the lesion: preferred site to collect the sample, because of the presence of more bacilli
- Biopsy from nerves or nodular lesions

### On staining with modified ZN stain:

- M. leprae; appear pink (AF), singly present or in groups (Cigar like bundles) bound together by glia to form globi inside macrophage
- Bacteriological index
- Morphological index

Are calculated based on microscopy

| Bacteriological index | Morphological index |
|---|---|
| Total number of AFB seen | We calculate, proportion of live bacilli in the smear |
| If <1 – paucibacillary<br>If >1 – multibacillary | Live bacilli – will take up stain ly/regularly stained<br>Example:<br>Total number of AFB =4<br>Total number of live AFB = 4<br>$MI = \frac{live}{Total}$<br>$= \frac{4}{4} = 1$ |
| It is a diagnostic index<br>It is used to classify leprosy | MI – falls with treatment<br>Therefore, it is a prognostic index<br>Eg. If after treatment, 3 out of 4 live bacilli die, then:<br>$MI = \frac{4-3}{4} = \frac{1}{4} = 0.25$ |

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2) Animal models – for research
   - Foot pad of mice
   - 9-banded armadillo (best)

## Lepromin test 00:29:27

- It is a type-IV Hypersensitivity reaction
- It is an assessor of CMI
- 0.1ml of lepromin Ag is injected intradermally to inner forearm
- Reading is taken two times:

| **After 48-72 hours** | **After 3 weeks** |
|---|---|
| Early reaction/ Fernandez reaction | Late reaction/mitsuda reaction |
| It is induration surrounded by erythema at the site of inoculation | Positive reaction:<br>Nodule >5mm in size |
| Area >10mm in diameter positive test | It can ulcerate later |

# ATYPICAL MYCOBACTERIA

## Atypical Mycobacteria 00:00:08

- They are called environmental mycobacteria / mycobacteria other than tubercle bacilli (MOTT).
- Runyon classified these MOTT, based on pigment production and rate of growth:

1.Photochromogens – Agents which produce pigments in the presence of light

Example: -

- Mycobacterium marinum – causes swimming pool granuloma or fresh tank granuloma
- Mycobacterium kansassi– causes lung disease resembling Tb.

2.Scotochromogens – agents which produce pigments in the presence and absence of light

Example:

- M. scrofulaceum – cause scrofula in kids – cervical lymphadenopathy
- M. szulgai – causes pulmonary disease and bursitis
- M. gordonae – also known as tap water bacillus
  - It is rarely isolated from pulmonary disease
- M.szulgai – at 37°c: scotochromogen
  - at 25°c: photochromogen

3.Non-chromogens – Do not produce any pigment

**Example:**

i. Mycobacterium avium – intracellulare (MAI) complex: It includes two species:

- M. avium: It is called as Battey bacillus (isolated from birds)
- M. intracellulare
- These two-cause opportunistic infection in HIV patients when CD4 + T cell count <50/microliter

  Diseases:
  - Lymphadenitis
  - Pulmonary disease
  - Disseminated disease

ii. M. xenopi:

- It has been obtained from hospital water supplies
- It can cause occasional nosocomial outbreaks
- It can cause occasional pulmonary disease in HIV patients

iii. M. ulcerans:

- It causes buruli's ulcer
- It produces an exotoxin known as mycolactone
- Only mycobacterial species which produces an exotoxin

iv. M. paratuberculosis/John's bacillus:

- It is suspected to play role in crohn's disease (?)
- It usually causes disease in cattle

## Rapid growers 00:10:07

- They grow on LJ medium within a week

  Example:

  - M. fortuitum
  - M. chelonae

  Both these causes post – trauma injection abscess and catheter related infection

  - M. abscessus: cause pulmonary infection
- Rapid growers show positive aryl sulfatase test.

# SUBCUTANEOUS MYCOSES –
# CHROMOBLASTOMYCOSIS

## Chromoblastomycosis 00:00:12

- Exogenous fungal infection
- Male > Female
- Lower limbs > upper limbs
- Mode of infection: Traumatic inoculation
- Chronic slow growing infection
- Lesions: polymorphic
- Most common type – Verrucous/cauliflower type/warty

  Other types: nodular and ulcerative lesions
- Caused by Dematiaceous/phaeoid fungi-Fungi have black/brown hyphae.
- Eg. Fonsecaea pedrosoi

  Fonsecaea compacta

  Phialophora verrucosa

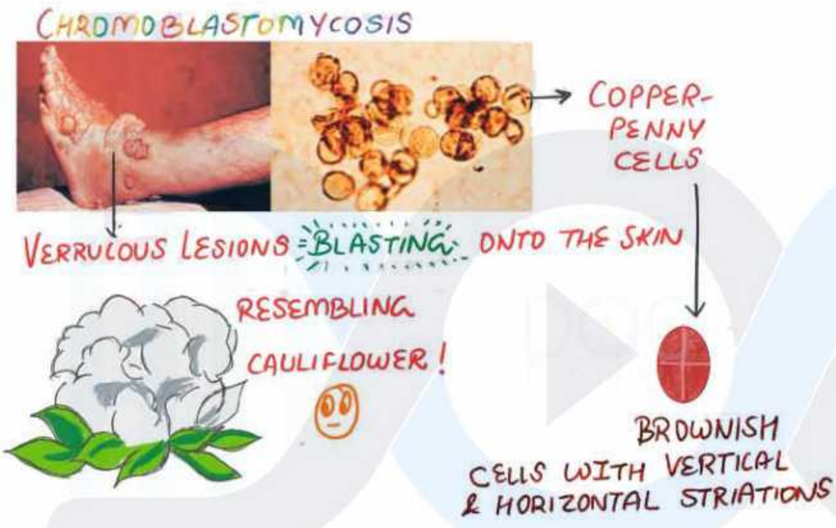
  Cladosporium carrionii

  Rhinocladiella aquaspersa

## Diagnosis:

1. Biopsy specimen: - spherical cells with internal septations:

   "copper penny cells"/"Medlar bodies"/"Muriform cells"/ "Sclerotic bodies"


CHROMOBLASTOMYCOSIS
COPPER-PENNY CELLS
VERRUCOUS LESIONS
BLASTING
ONTO THE SKIN
RESEMBLING CAULIFLOWER !
BROWNISH CELLS WITH VERTICAL & HORIZONTAL STRIATIONS

Chromoblastomycoses Fig: 1

## Treatment:

- Surgical removal – Cryosurgery/Laser surgery followed by Antifungals (Itraconazole).

# SUBCUTANEOUS MYCOSES - SPOROTRICHOSIS

## Sporotrichosis 00:00:23

- Caused by Sporothrix schenckii
- Sporothrix schenkii
  - Dimorphic fungus
  - Causes subcutaneous infection
  - Present on woods & plants
  - Exogenous infection
  - Mode of infection: Traumatic inoculation into the skin
  - The fungi secretes enzymes – Aspartate proteinase, serine proteinase
  - These enzymes help in invasion of the fungus into the tissues
- Most common clinical form: Lymphocutaneous sporotrichosis

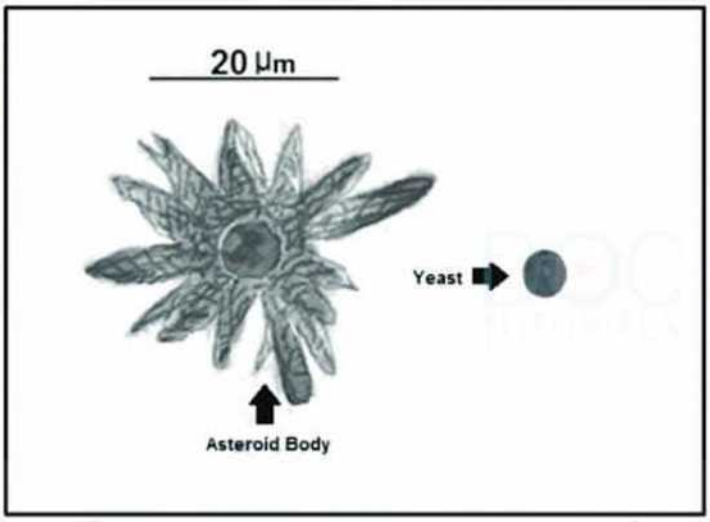
Fungus enters the skin
↓
Subcutaneous tissue
↓
Invades lymphatic vessels
↓
Lymphangitis
↓
Fungus comes out of the inflamed lymphatics
↓
Ulcers in series/rows

- Disease is also known as rose picker's disease or rose gardener's disease
- In India – prevalent in sub-himalayan region
- M > F

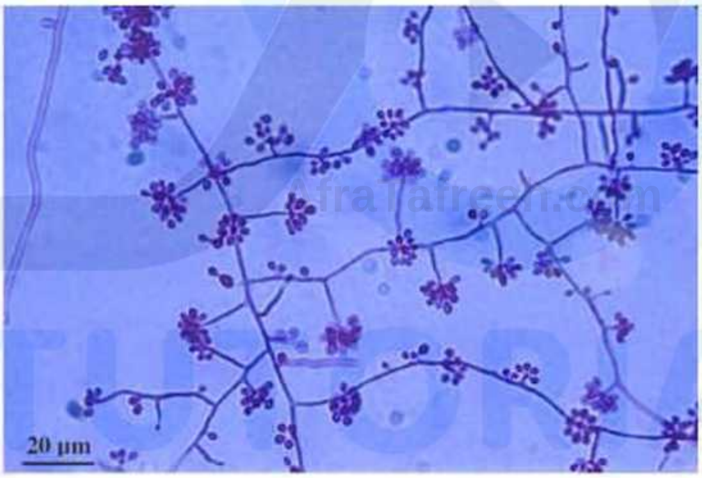
## Diagnosis:

- Specimen:
  - Swab from ulcer
  - Pus/aspirate from nodules

1. KOH mount or calcofluor white staining
   - Elongated yeast cells (3-5 µm)
2. Biopsy of tissue
   - Cigar shaped yeast cell in center
   - Star shaped host reaction surrounding the yeast cell (Ag-Ab complex) – eosinophilic
   - Asteroid body/ Splendore-Hoeppli phenomenon
3. Definitive method – Culture
   - At room temperature:
     - Mold form
     - Rosette arrangement of microconidia


20 µm
Yeast
Asteroid Body

“Yeast form”-sporotrichosis Fig: 1



“Mold form”-sporotrichosis Fig: 2

## Treatment:

- DOC for all forms of sporotrichosis: **Itraconazole**
- For disseminated sporotrichosis – **Amphotericin-B.**

# SUBCUTANEOUS MYCOSIS – MYCETOMA

## Mycetoma/Madura Foot/Maduramycosis 00:00:10

- 1st identified in Madurai by Sir John Gill
- Chronic granulomatous fungal infection
- Triad of – Tumefaction of the affected tissue
  - Multiple discharging sinuses
  - Sulphur granules/grains
- Sulphur granules – sulphur absent
  - Microcolonies of organisms present
- Exogenous infection
- Mode of infection – Traumatic inoculation
- Male > Female
- Lower limbs > upper limbs

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**Mycetoma:**

- **Eumycetoma**
  - Caused by fungi
    - **Dematiaceous fungi (black/brown/ hyphae)**
      - Black granules
    - **Hyaline fungi** (colorless / transparent hyphae)
      - White granules
- **Actinomycetoma**
  - Caused by bacteria (G + Filamentous bacteria)

- Dematiaceous fungi: - Madurella mycetomatis
  - Madurella grisea
  - Exophiala jeanselmei
  - Curvularia
- Hyaline fungi – Pseudallescheria boydii
  - Acremonium species
- Actinomycetoma – Actinomadura madurae
  - Actinomadura pelliteri → pink red granules
  - Nocardia asteroides
  - Nocardia brasiliensis
  - Nocardia caviae
  - Streptomyces somaliensis

- Except A.pelliteri, all other causes of actinomycetoma produce white to yellow granules.

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### Diagnosis:

i. Macroscopy: Color
Size
Texture of granules

ii. Staining, culture – Granules crushed between 2 slides

### Staining:

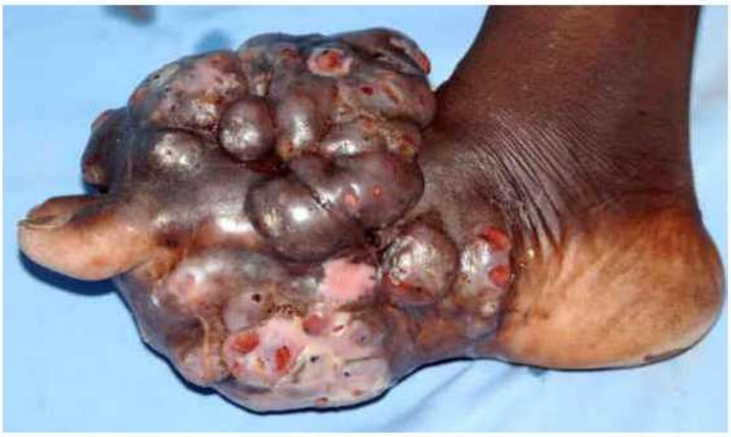
- Eumycetoma: KOH mount
  - Fungal hyphae (2-6 microns wide)
- Actinomycetoma – Gram stain: Gram +ve filaments (0.5 – 1 microns wide)

- Nocardia: Modified Acid-Fast stain

### Culture: On SDA

- Prognosis:
  - Actinomycetoma: Better prognosis
    Responds to sulfonamides.
  - In India: Actinomycetoma > Eumycetoma
  - Eumycetoma – Poor prognosis
    - Rarely responds to antifungals
    - Amputation of limb

| | Eumycetoma | Actinomycetoma |
|---|---|---|
| 1. Tumor | Single lesion<br>Well defined margins | Multiple lesions<br>Ill defined margins |
| 2. Sinuses | Few<br>Appear Late | Multiple<br>Appear Early<br>Raised Inflamed openings |
| 3. Discharge | Serous | Purulent |
| 4. Bone | Osteosclerotic lesions | Osteolytic lesions |
| 5. Color of Granules | Black/White | Whitish-yellow/pink |
| 6. Grains have | Fungal filaments (>2microns) | Filamentous bacteria (<2microns) |



Mycetoma Fig: 1

# SUBCUTANEOUS MYCOSES – RHINOSPORIDIOSIS

## Rhinosporidiosis 00:00:16

- Caused by Rhinosporidium seeberi
- Not a fungus
- Regarded as Mesomycetazoan – Aquatic protistan organism (in stagnant water)
- Non cultivable organism
- Most common clinical form – Nasal Polyp (figure 1)

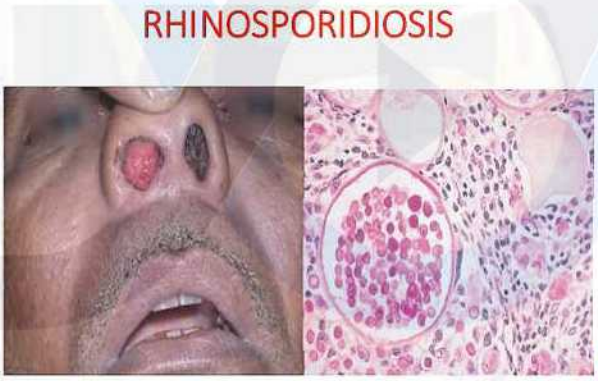
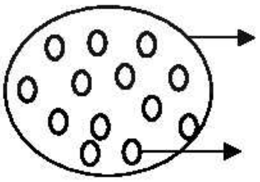

RHINOSPORIDIOSIS

Fig: 1

- Endosporulating spherule
- Microscopy: Spherule / Sporangium


Spherule / Sporangium (350µm)
Endospores (6–9µm)

Spherule stains well with Meyer's Mucicarmine stain

- Mode of infection: Inhalation of spores present in stagnant water
- Epidemiology: India – Kerala, Tamilnadu, Srilanka

## Treatment:

- Surgical excision with cauterisation
- Dapsone – Effective in some cases
- Recurrence is common
- Rare sites of Infection:
  - Conjunctiva
  - Ears
  - Larynx
  - Bronchus
  - Genitalia

# SYSTEMIC MYCOSES – HISTOPLASMOSIS

## Systemic Mycoses 00:00:10

- Caused by Thermally dimorphic fungi
- Known as Endemic mycoses
- Mode of infection: Inhalation of spores/hyphae
- Mostly asymptomatic
- In people with less immunity: Symptomatic
- No man-to-man transmission

## Histoplasmosis 00:02:20

- Also known as Darling's disease
- Caused by Histoplasma capsulatum – it is not capsulated
- Mode of infection – Inhalation of microconidia (2-4 μm)
- Source of infection – soil contaminated with bird droppings (birds- starlings) & bat faeces

### Epidemiology:

- Endemic in: Central & Eastern states → In Ohio & Mississippi river valleys.

Microconidia are inhaled

↓

Reach lungs

↓

Microconidia are eaten by alveolar macrophages

↓

Microconidia change into yeasts

- Intracellular yeasts – reach local lymph nodes/ other sites via blood

## Diagnosis:

Specimen: Sputum

Aspirates from liver, spleen, bone marrow

i. Microscopy: Giemsa stain:

- Intracellular fungus

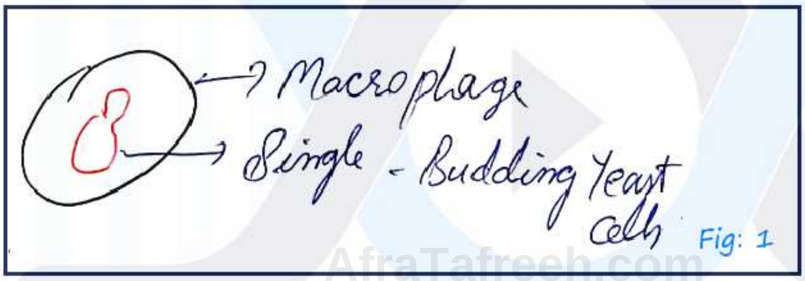

Macrophage
Single - Budding Yeast cell

Fig: 1

ii. Culture on SDA: Incubated at 25°C

- **Mold form at 25°C** → white to buff brown colonies

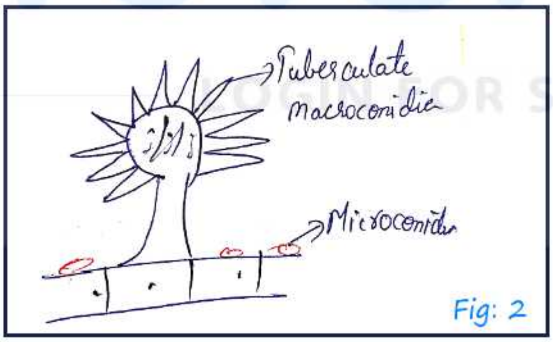

Tuberculate macroconidia
Microconidia

Fig: 2

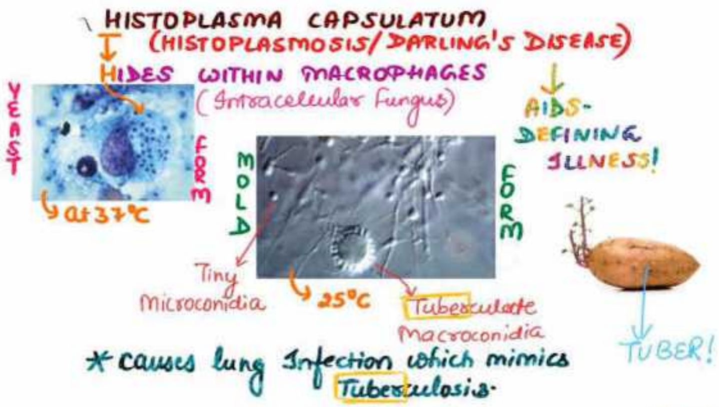

HISTOPLASMA CAPSULATUM
(HISTOPLASMOSIS/DARLING'S DISEASE)
HIDES WITHIN MACROPHAGES
(Intracellular Fungus)
AIDS-DEFINING ILLNESS!
YEAST FORM
at 37°C
MOLD FORM
Tiny Microconidia
25°C
Tuberculate Macroconidia
TUBER!
* causes lung Infection which mimics Tuberculosis.

Fig: 3

Histoplasma capsulatum

- Thin, hyaline, septate hyphae.

iii. Yeast conversion test:

- Subculture of mold form onto BHI – Agar/Kelly's medium, and incubated at 37°C – **Creamy colonies**
- Microscopy – Yeast form

iv. Exo-antigen test:

- Mold form is mixed with water
- Water-soluble antigens come out
- Antiserum is then added → If positive test → **Histoplasma capsulatum**

v. Ag detection:

- Polysaccharide antigens of cell wall of H.capsulatum are detected by ELISA

vi. Ab detection – CFT and Immunodiffusion test

# SYSTEMIC MYCOSES - BLASTOMYCOSIS

## North American Blastomycosis/Chicago Disease / Gilchrist's Disease 00:00:14

- Caused by Blastomyces dermatitidis
- Mode of infection: Inhalation of microconidia

↓

Reach alveolar macrophages

↓

Engulfed by macrophages

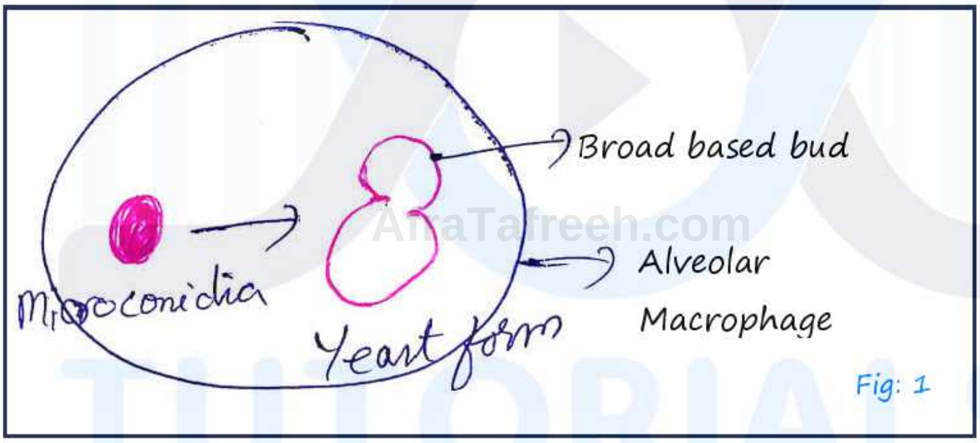

Broad based bud
Microconidia
Yeast form
Alveolar Macrophage

Fig: 1

### Yeast form:

- Expresses a protein BAD-1 (Blastomyces Dermatitidis Adhesin-1)
- BAD - 1: Important virulence factor

  Induces cellular & humoral immune response
- Most infections - asymptomatic
- Most common extrapulmonary manifestation: Skin disease
  - Verrucous lesions

## Treatment:

i. Oral itraconazole – Progressive lung disease
- Chronic suppression in AIDS patients

ii. Parenteral Itraconazole/Amphotericin B-In disseminated infections

iii. Fluconazole – for meningitis

  - Ulcerative lesions
- In 1/4th of infections – **Osteomyelitis**
- In HIV infected people – **Brain abscess**
- Epidemiology: North and Eastern US (Ohio & Mississippi river valleys)

## Diagnosis:

Specimen: Biopsy (37°c)

i. Histopathological examination: yeast form – broad based budding: "figure of 8 – appearance"
  - can be intracellular/extracellular
  - 5-15 microns

ii. Culture of specimen at 25°C
  - Mold/hyphal form
  - No macroconidia
  - Only microconidia

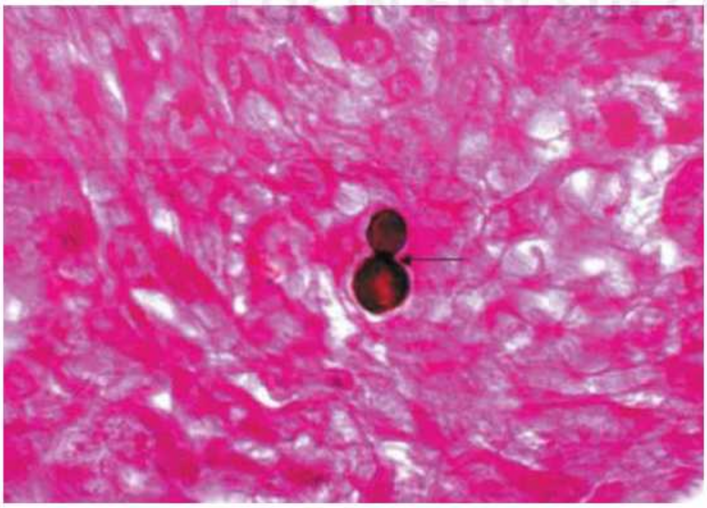
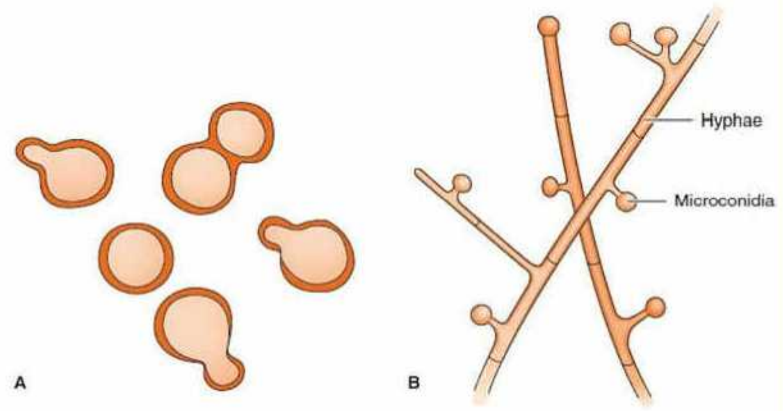


Fig: 2

Blastomyces dermatitidis – broad based budding



Yeast with a broad-based bud Fig: 3

iii. Yeast conversion test

iv. Exo-antigen test

### Treatment:

i. Oral Itraconazole mostly

ii. Disseminated cases – Amphotericin – B

iii. Surgical Excision.

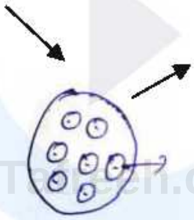
# SYSTEMIC MYCOSIS - COCCIDIOIDOMYCOSIS

## Coccidioidomycosis 00:00:10

- Caused by Coccidioides immitis
- Endemic in arid regions of South-west US and Latin America
- Disease- Desert Rheumatism/Valley fever
- Mode of infection: Inhalation of Arthroconidia (2-4microns)

↓ (Barrel shaped)

In lungs, they change into SPHERULES (30-120 microns)

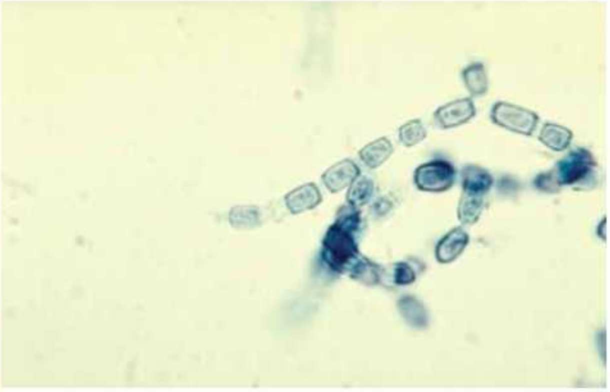


Rupture of spherule – endospores released

↓

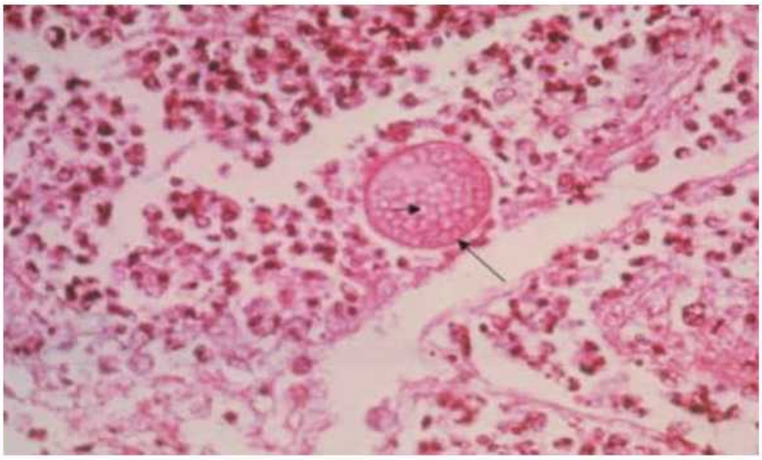
Endospores transform into spherule

- At 37°C → Endosporulating spherules



**LPCB mount → Arthrospores** Fig: 1

- Empty cells are present between arthrospores



Endosporulating spherule Fig: 2

- Granulomatous lesions – most commonly in bones & CNS (Meningitis)

## Diagnosis:

- Specimen: Biopsy
  - i. HPE: Endosporulating spherules
  - ii. Culture at 25°C – mold form
  - iii. Yeast conversion test – tough to do
  - iv. Exo-antigen test

## Treatment:

- Itraconazole/Amphotericin – B – for persistent lung lesions

  – Disseminated disease
- Fluconazole – for meningitis.

# SYSTEMIC MYCOSES –

# PARACOCCIDIOIDOMYCOSES

## Paracoccidioidomycosis 00:00:10

- Also known as South American Bastomycosis
- By Paracoccidioides brasiliensis
- "Lutz mycosis"
- Epidemiology: Endemic in Latin America
- Mode of infection: Inhalation of spores

  ↓

  Involvement of oral cavity, cervical lymph nodes
- Mostly asymptomatic
- In immunocompromised people: Disseminated disease

### Diagnosis:

- Specimen: BAL

  Sputum

  Biopsy specimen

i. HPE of biopsy at 37°C

  - Multiple budding yeast cells

    ↓

  - Captain's wheel appearance/ Mariner's wheel appearance/Pilot's wheel appearance:

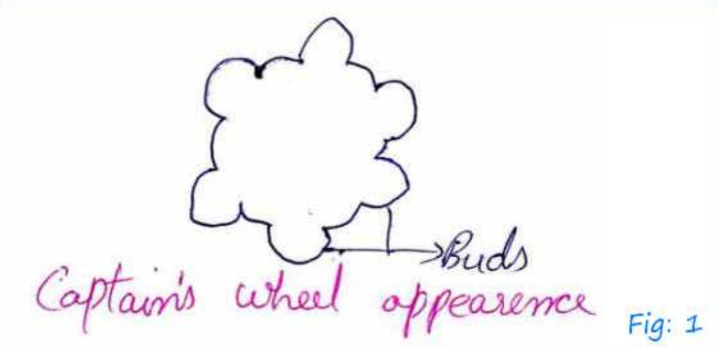
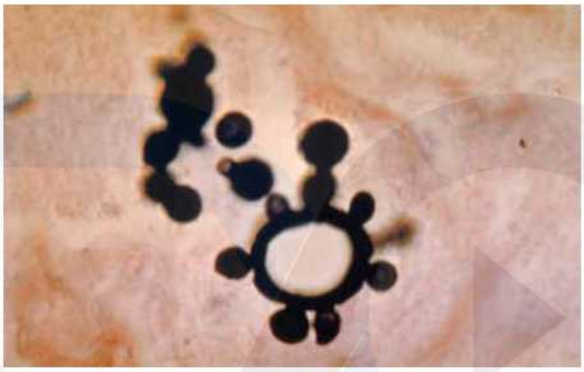

Buds
Captain's wheel appearence

Fig: 1

- Only 2 buds → Mickey mouse appearance



Paracoccidiodies brasiliensis Fig: 2

ii. Culture at 25°C: Hyaline, septate hyphae with chlamydospores

## Treatments

- Oral Intraconazole

# SYSTEMIC MYCOSES – PENICILLIOSIS

## Penicilliosis 00:00:07

- Caused by Penicillium marneffei/Talaromyces marneffei
- Only species in Genus which is dimorphic
- Epidemiology: South China
  Southeast Asian countries
  Eastern states in India (Manipur)
- AIDS – defining illness
- Infects Reticuloendothelial system
- Mode of infection: Inhalation of spores
- Mostly asymptomatic
- Disseminated infection in Immunocompromised people (HIV-infected)
- **Reservoir: Chinese Bamboo Rat**

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### Diagnosis:

- Specimen – biopsy
  - i. HPE of biopsy: yeast form at 37°C
  - ii. Culture at 25°C – mould form
    - Brush border/broom like appearance of microconidia
    - **Mould form – produces red-wine colored pigment**

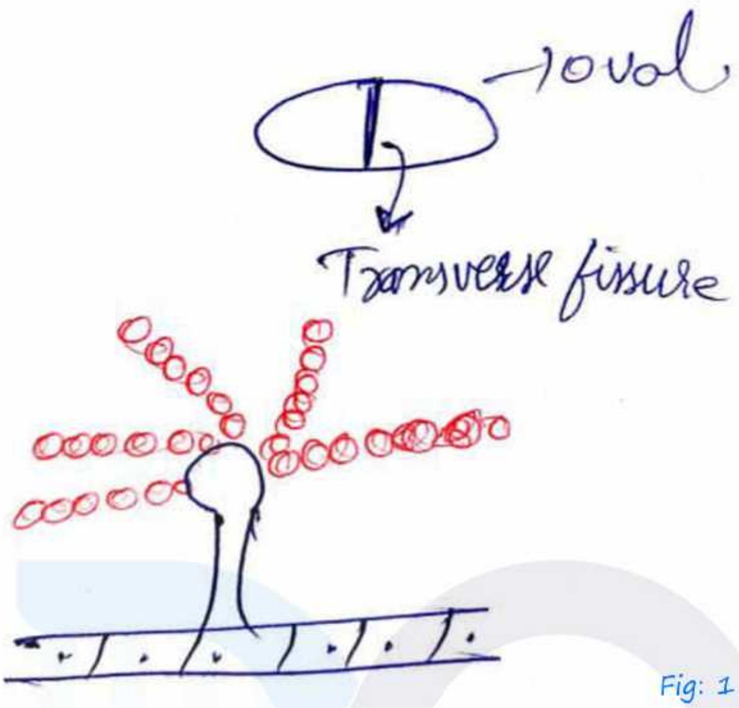
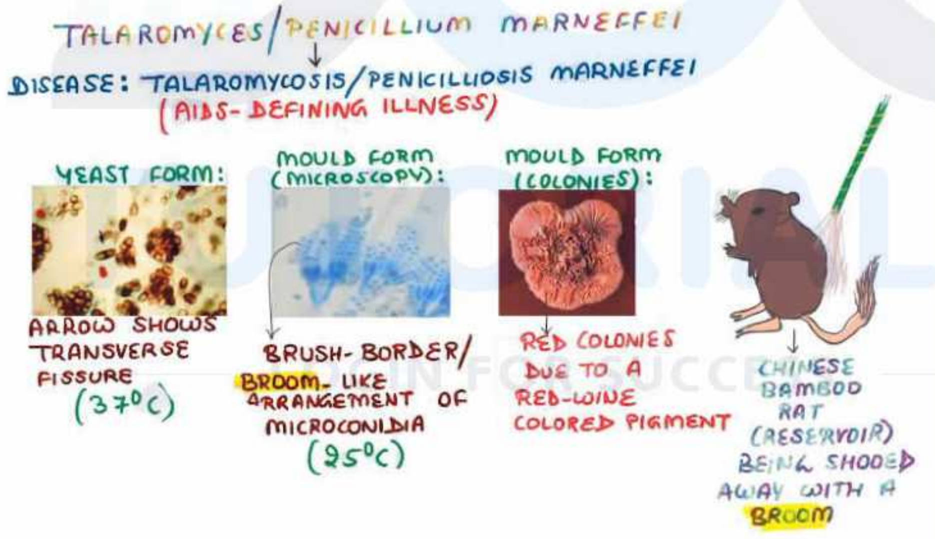

oval
Transverse fissure

Fig: 1


TALAROMYCES/PENICILLIUM MARNEFFEI
DISEASE: TALAROMYCOSIS/PENICILLIOSIS MARNEFFEI
(AIDS-DEFINING ILLNESS)
YEAST FORM:
ARROW SHOWS TRANSVERSE FISSURE
(37°C)
MOULD FORM (MICROSCOPY):
BRUSH-BORDER/BROOM-LIKE ARRANGEMENT OF MICROCONIDIA
(25°C)
MOULD FORM (COLONIES):
RED COLONIES DUE TO A RED-WINE COLORED PIGMENT
CHINESE BAMBOO RAT (RESERVOIR) BEING SHOOED AWAY WITH A BROOM

Summary Penicillium marneffei Fig: 2

## Treatment:

i. Mild Disease: Itraconazole x 12 weeks

ii. In AIDS patients: Amphotericin-β – till condition improves

↓ followed by

Itraconazole maintenance therapy x 12 weeks

# OPPOURTONISTIC MYCOSES – CANDIDIASIS

## Candidiasis/Moniliasis 00:01:38

- Caused by genus Candida – yeast like fungi
- Candida albicans: most common cause of infection in humans
- Commensal on – Skin
  - Oral cavity
  - GIT
  - Genitourinary tract
- Risk factors – HIV Infection
  - Steroids/other immunosuppressants
  - Neutropenia
  - Alcoholism
  - Leukemia
  - Zinc deficiency

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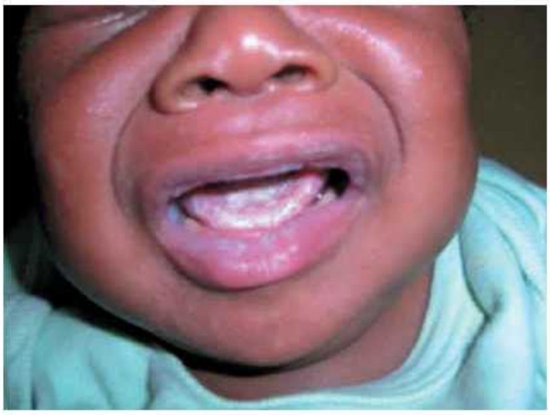
### Virulence factors of C.albicans

1. Adhesins: Attachment of fungus to host skin & mucus membrane
2. Enzymes: Aspartate proteinase
   Serine proteinase
3. Toxins: Glycoprotein extracts from cell are pyrogenic (≈ Bacterial endotoxin)
4. Pseudohyphae: Indicate tissue invasion



Candida albicans-Oral thrush Fig: 1

## Clinical Features:

1. Oral thrush/Oral candidiasis: White patches (painless) – adhered to oral mucosa (figure 1)
2. Esophageal candidiasis (AIDS defining illness)
3. Vaginal candidiasis – in diabetic women
   - Thin vaginal discharge – initially
     curdy white discharge – later
   - Intense pruritus
   - Vaginal $P^H < 4.5$
4. Intertrigo: infection of folds of skin
   - Erythema & pustules present
5. Infective endocarditis
6. Candidemia
7. Urinary tract infection

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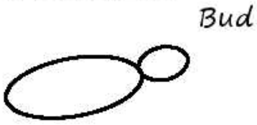
## Diagnosis:

- Specimen: collected depending on clinical features

1. Wet mount:



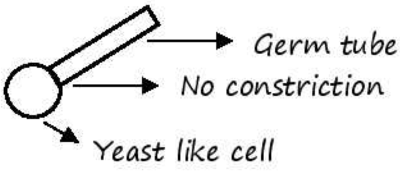
- Budding yeast like cells

2. Gram stain: gram +ve yeast-like cells are seen
   - In wet mount & Gram stain, if pseudohyphae are present → It indicates tissue invasion
3. Culture on SDA:
   - Creamy white, pasty colonies (figure 2)
   - Appear in 2-4 days

Candida colonies on SDA Fig: 2

4. Germ tube test/Reynolds braude phenomenon:

Colony + human serum
↓
Incubated for 2 hours at 37°C
↓
Under microscope



- C.albicans – non-thermally dimorphic
- Germ tube is a true hypha

- Germ tube test – positive in Candida albicans
  Candida dubliniensis

5. Growth at 45°C
   - C. albicans – grows
   - C. dubliniensis – does not grow

6. Chromagar medium:
   - C. albicans – produce light green colored colonies.

7. Cornmeal agar – C. albicans form Chlamydospores

8. Carbohydrate assimilation test
   Carbohydrate fermentation test ] – Species identification

9. Antibody detection: ELISA
   - Detects Abs against cell wall mannan

10. Antigen detection: ELISA & Latex agglutination
    - Cytoplasmic antigens
    - Cell wall- Mannan antigen

11. G-Test: Detects β-1-3-D glucan

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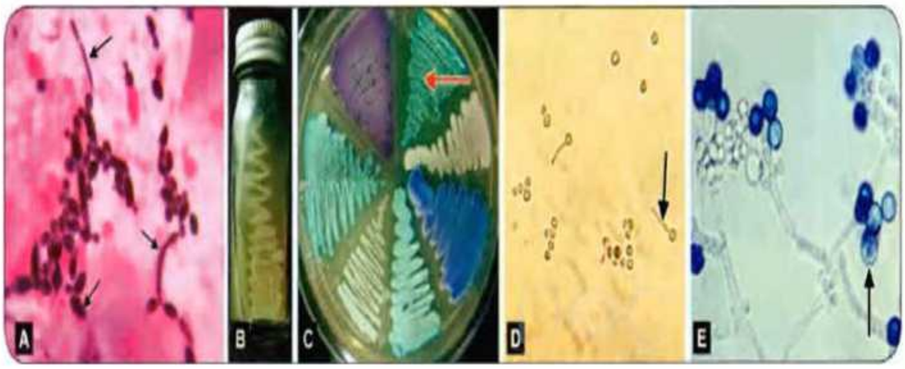
## Treatment:

1. Cutaneous candidiasis and oral thrush – topical azoles (DOC)
2. Esophageal and vaginal candidiasis: DOC: Oral fluconazole or Caspofungin
3. Disseminated candidiasis: Liposomal amphotericin-'B' or caspofungin

C. krusei
C. glabrata ] – Intrinsically resistant to azoles



Candida albicans Fig: 3

Figure 3: Candida albicans-gram-positive oval budding yeast cells with pseudohyphae; B. Candida albicans on SDA shows creamy white colonies; C. CHROMagar showing colonies of various Candida species producing different colors (e.g. light-green color by C. albicans, red arrow); D. Candida albicans shows positive germ tube test (arrow showing); E. Candida albicans shows thick walled chlamydospores (arrow showing)

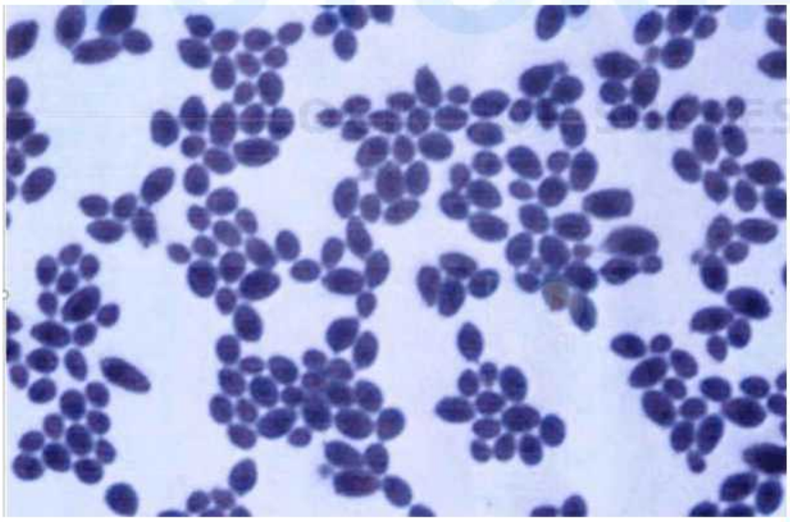


Fig: 4

Candida on Gram stain

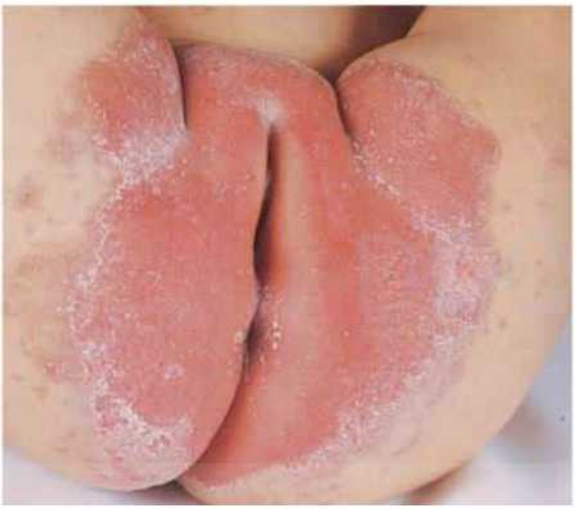


Fig: 5

Diaper rash by C.albicans

# OPPORTUNISTIC MYCOSES - ASPERGILLOSIS

## Aspergillosis 00:00:10

- By genus Aspergillus
- Mold/filamentous fungus with septate hyphae
- Acute angled/dichotomous branching of hyphae
- Saprophytes
- Important species:
  - A. fumigatus → most common species infecting humans
  - A. flavus→ Nosocomial pathogen
  - A. niger
- Mode of infection: Inhalation of spores/hyphal forms
  - Most common mode
- Risk factors: Neutropenia
- NOTE: In HIV: CD4 +ve T-cells/$T_H$ cells are affected

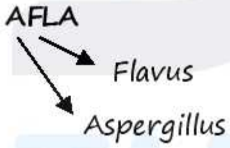
  Neutrophil count- remains normal until late stages of disease so Aspergillosis is uncommon.

### Clinical Features:

1. Allergic Bronchopulmonary Aspergillosis [ABPA]:
   - In patients with cystic fibrosis, asthma
   - Characterized by – Pulmonary infiltrates Chest x-ray
     - Central Bronchiectasis
   - Wheezing present
   - Increased IgE in blood against specific species

- Eosinophilia

2. Aspergilloma/Fungal Ball:
   - Aspergillus colonises the lung cavity (Tb, Cancer)
3. Invasive pulmonary aspergillosis:
   - In people with profound neutropenia or Immunocompromised people
   - Diffuse pulmonary infiltrates
   - HALO sign
   - AIR CRESCENT sign
4. Otitis externa: A. niger
5. Keratomycoses – In farmers during harvesting season
6. Aflatoxicosis: Not infection, its intoxication



- A. flavus produces Aflatoxins which are hepatotoxic
- Contaminates stored grains – Aflatoxins are produced in humid conditions

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## Diagnosis:

1. Mainstay of diagnosis: HPE of biopsy specimen
   - Stains with H & E stain

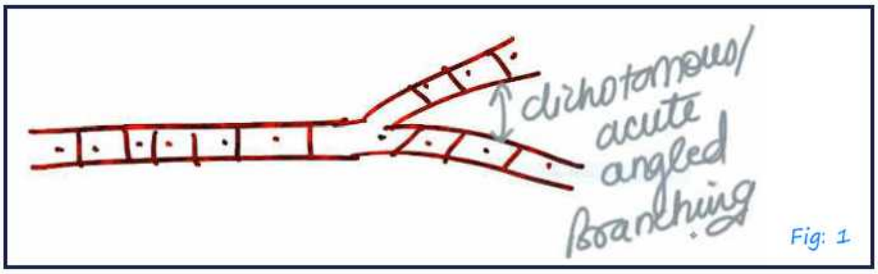
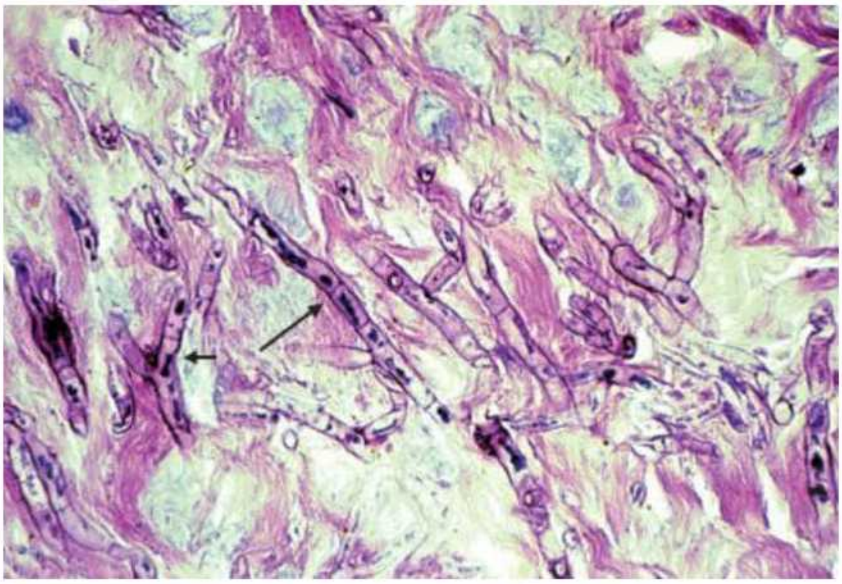


Fig: 1



Aspergillus Hyphae

Fig: 2

2. Culture: Hyphal form

3. Microscopy:

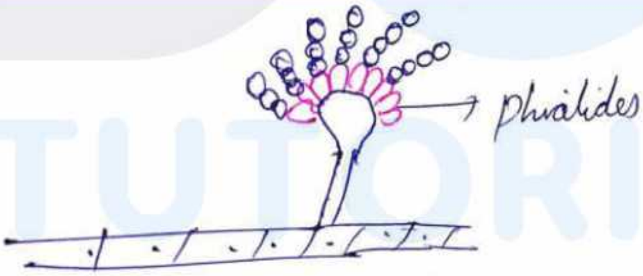


Fig: 3

- Phialides – cells which give rise to conidia

  - Present in 1 or 2 rows

- If 2 rows present: 1st row is called metulae

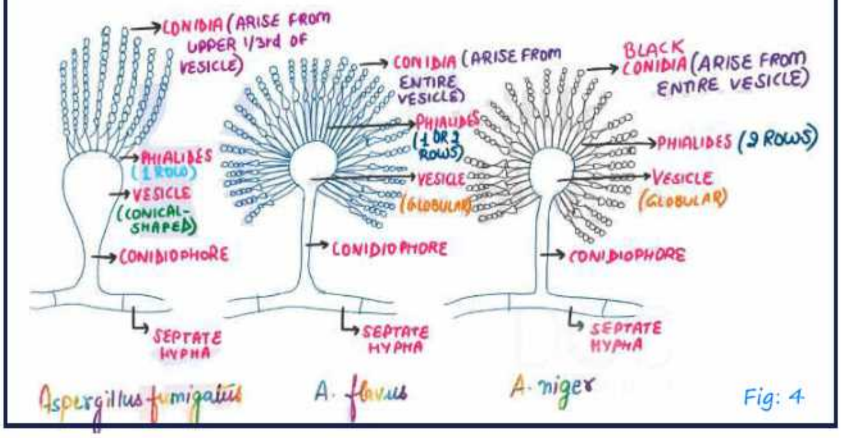
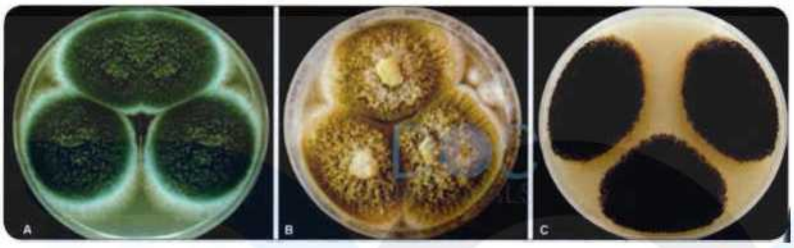

CONIDIA (ARISE FROM UPPER 1/3rd OF VESICLE)
PHIALIDES (1 ROW)
VESICLE (CONICAL-SHAPED)
CONIDIOPHORE
SEPTATE HYPHA
Aspergillus fumigatus
CONIDIA (ARISE FROM ENTIRE VESICLE)
PHIALIDES (1 OR 2 ROWS)
VESICLE (GLOBULAR)
CONIDIOPHORE
SEPTATE HYPHA
A. flavus
BLACK CONIDIA (ARISE FROM ENTIRE VESICLE)
PHIALIDES (2 ROWS)
VESICLE (GLOBULAR)
CONIDIOPHORE
SEPTATE HYPHA
A. niger

Fig: 4



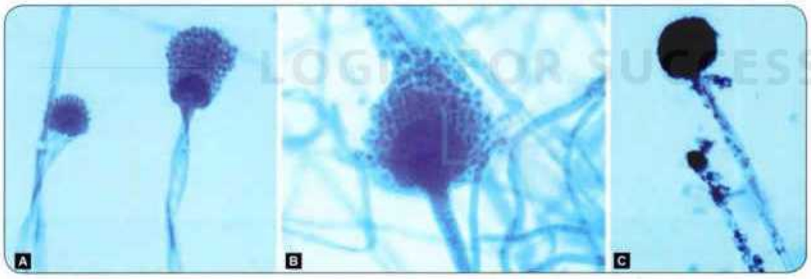
Aspergullus colonies

Fig: 5

a. A. fumigatus – smoky green, reverse – white colonies

b. A. flavus– yellow green colonies, reverse – white

c. A. niger – black colored colonies, reverse – white



LPCB MOUNT

Fig: 6

a. A. fumigatus

b. A. flavus

c. A. niger

**Treatment:**

- Invasive disease: Voriconazole
- ABPA: Itraconazole
- Chronic pulmonary aspergillosis: Itraconazole/Voriconazole

# OPPORTUNISTIC MYCOSES – ZYGOMYCOSIS

## Zygomycosis/Phycomycosis 00:00:10

- Caused by members of class Zygomycetes/Phycomycetes
- Important genera: Rhizopus
  Absidia
  Mucor
- Most common species: Rhizopus arrhizus
- Risk factors: Diabetic ketoacidosis
  Desferrioxamine therapy
- Saprophytes
- Mode of infection: Inhalation of spores
- Most dreaded disease: **Rhinocerebral mucormycosis**
- Angioinvasive fungus

NOTE: Aspergillus is also an angioinvasive fungus.

### Diagnosis:

Biopsy specimen

i. HPE with H & E stain: **Broad, Aseptate, Ribbon like, Irregular hyphae**

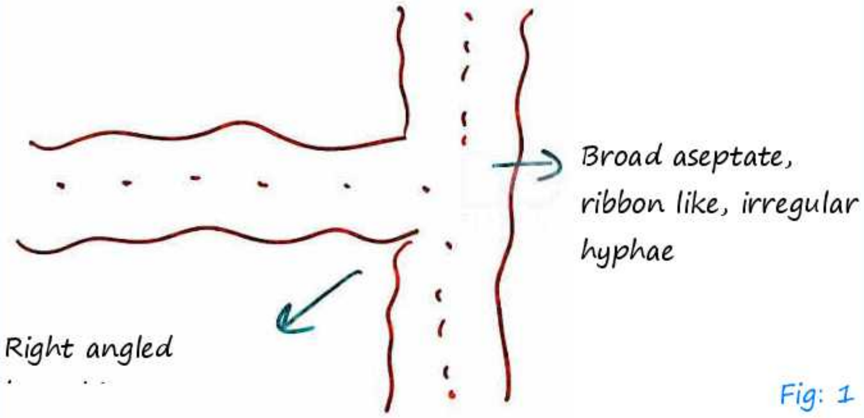
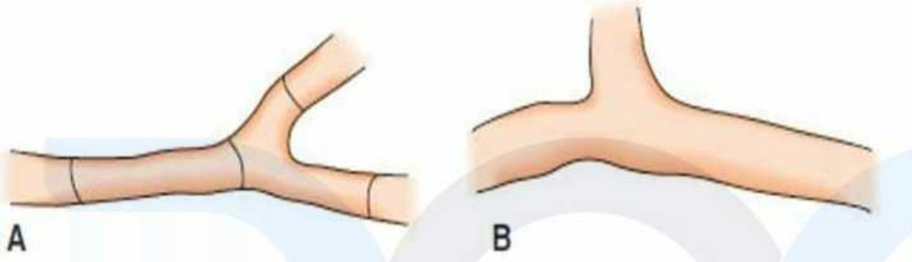
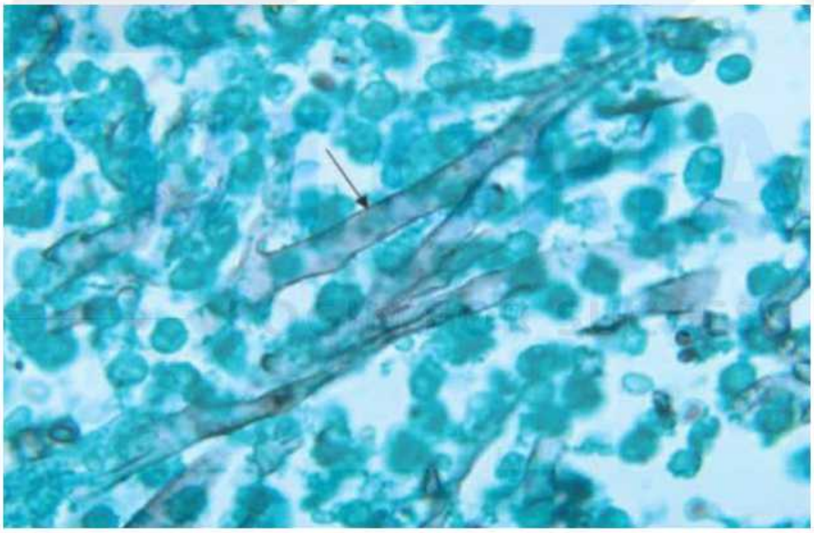

Broad aseptate, ribbon like, irregular hyphae
Right angled

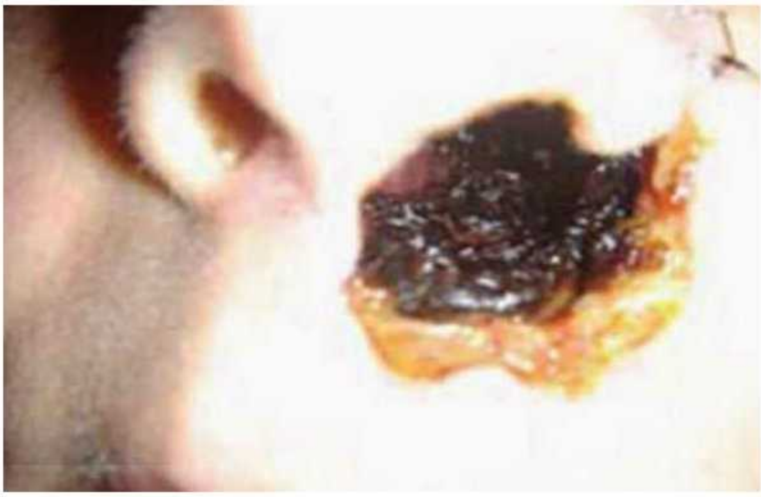
Fig: 1



A. Aspergillus B. Mucor Fig: 2



Mucor species Fig: 3



Mucormycosis Fig: 4

## Treatment

i. Rhinocerebral mucormycosis → Surgical debridement (mainstay)

DOC: Amphotericin – B

Alternate drugs: Posaconazole/Isavuconazole

A-Aspergillus B-Mucor

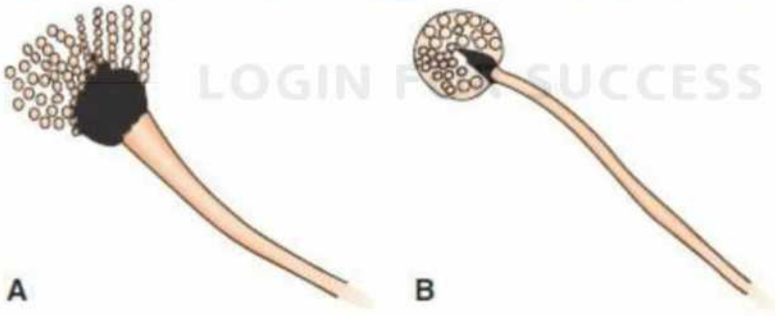


Fig: 5

# OPPORTUNISTIC MYCOSES – PNEUMOCYSTOSIS

## Pneumocystosis 00:00:10

- Caused by Pneumocystis jirovecii
- Earlier considered a protozoan; now a fungus
- Basis of classification of Pneumocystis jirovecii under fungi is Nucleic acid studies
- P.carinii → Rat species
- P.jirovecii → Non cultivable fungus
- Morphological forms → Cyst: spherical: has 8 intracystic bodies

  Trophozoite: Amoeboid form
- Cysts – Present in the environment
  - Infective form
  - Mode of infection → inhalation of cysts
- In humans: Both cysts + trophozoites are present
- Disease: pneumonia in:
  - Premature babies
  - HIV infected people, when $CD_4$ – T cell falls below 200/μm
- No disease occurs in Immunocompetent people.
- Alveolar macrophages – Principle host effector cells
- Fungus attaches to Type – I pneumocytes – remains extracellular & damages them
- Fungus – causes proliferation of type – II pneumocytes

- Surfactant abnormalities

- BAL fluid: Increased IL-8 and neutrophils
- Trophozoites – cause inflammatory response in the lungs

↓

plasma cells are recruited

↓

**Frothy exudate filling alveoli: Plasma cell pneumonia**

## Diagnosis:

- Best sample: BAL
- Sputum – Inferior sample

i. Gomori methanamine silver stain: **Crushed ping pong balls-appearance** of cysts against a greenish background. Cyst's stain black (figure 1)

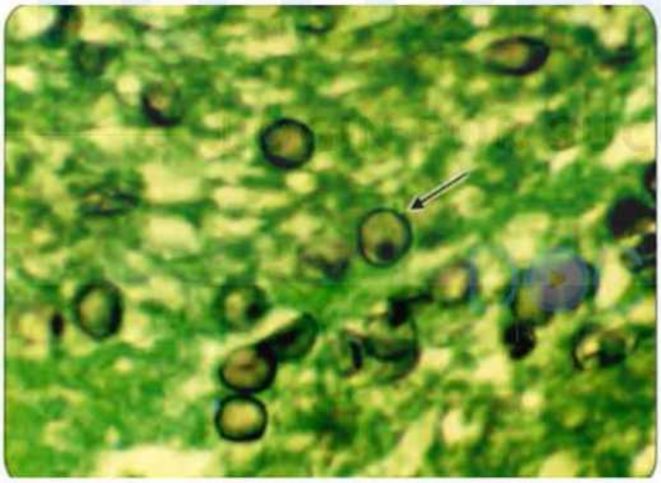


Fig: 1

ii. Giemsa stain:

- Stains intracystic bodies & not the cyst wall

iii. Direct fluorescent antibody (DFA) stain: The best stain

(Highly sensitive and specific stain)

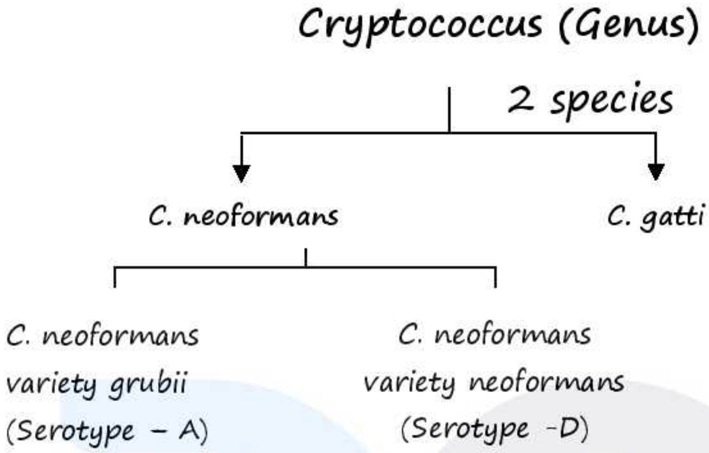
iv. Most sensitive and specific method of diagnosis: PCR

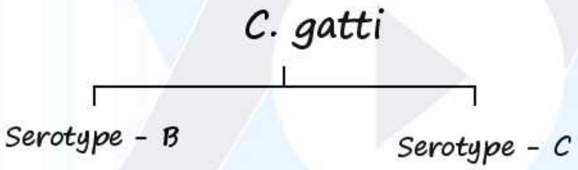
**Treatment:**

- Cotrimoxazole – both for treatment & prophylaxis

# OPPORTUNISTIC MYCOSES – CRYPTOCOCCOSIS

## Cryptococcosis/European blastomycosis 00:00:11


Cryptococcus (Genus)
2 species
C. neoformans
C. gatti
C. neoformans variety grubii (Serotype – A)
C. neoformans variety neoformans (Serotype -D)


C. gatti
Serotype – B
Serotype – C

- "Cryptococcus neoformans" – Encapsulated yeast
  - True yeast
  - Polysaccharide capsule
- Reservoirs → Eucalyptus trees
  → Soil contaminated with pigeon excreta
- Mode of infection: Inhalation
- Infective form: Aerosolized yeast cells, Basidiospores (sexual state)
- Most common site of infection: lungs
  Most infections are asymptomatic
- Fungus crosses BBB either- directly or "Trojan horse"
  – carried within macrophages

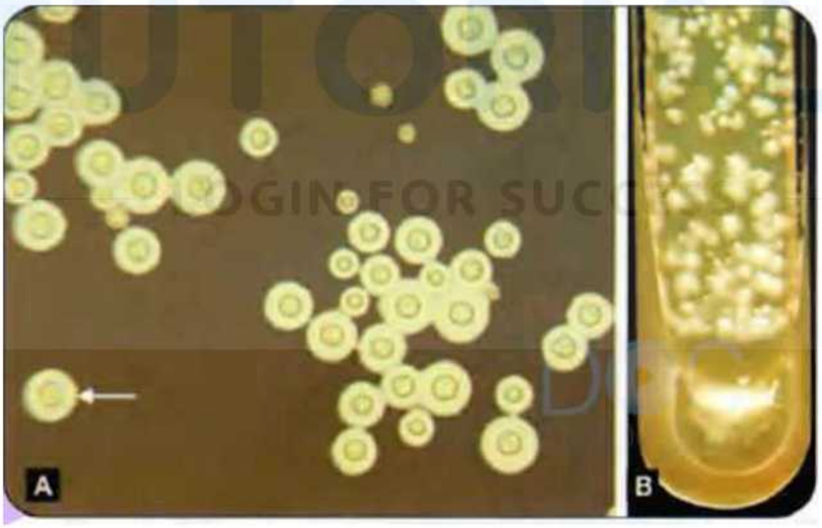
- Usually seen in immunocompromised people specially HIV-infected patients → Meningitis
  ↓
  (Subacute to chronic)
  Chronic headache

- Disseminated infection can occur to bones and skin
- Involvement of skin: "proof of disseminated infection"
- Virulence factors: i. Capsule – Polysaccharide
  Antiphagocytic
  ii. Ability to produce Melanin because of phenyl oxidase
  iii. Enzymes – Urease
  Phospholipase

## Diagnosis:

- Specimens: 1. CSF
  2. Serum

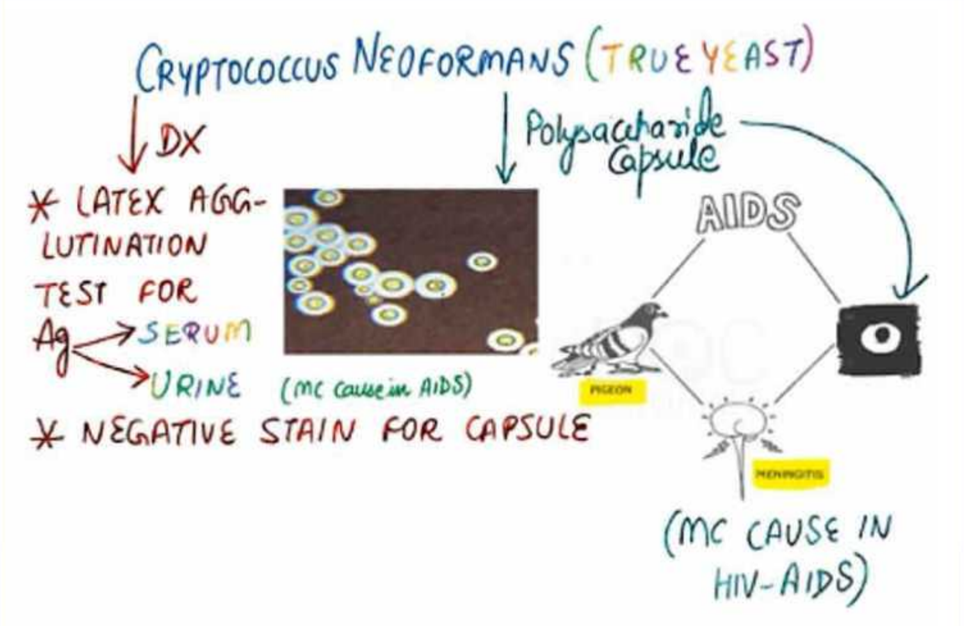
1.s SDA: Creamy white mucoid/shiny colonies



A. Negative stain of Cryptococcus

B. Culture of Cryptococcus on SDA

Fig: 1


CRYPTOCOCCUS NEOFORMANS (TRUE YEAST)
DX
* LATEX AGGLUTINATION TEST FOR
Ag → SERUM
→ URINE
(MC cause in AIDS)
* NEGATIVE STAIN FOR CAPSULE
Polysaccharide Capsule
AIDS
PIGEON
MENINGITIS
(MC CAUSE IN HIV-AIDS)

Summary of Cryptococcus neoformans Fig: 2

2.Culture on Bird seed agar } Black-brown colonies
Niger seed agar }

3.Urease test

4."Maltese crosse" appearance:

- Refers to the arrangement of capsular material seen under polarizing microscope

5.Assimilation of Inositol, Nitrate

6. Mouse pathogenicity test

**Treatment:**

- For Cryptococcosis without CNS involvement: DOC – Fluconazole

  Cryptococcosis with CNS involvement (foe example in HIV patients)

  ↓

  Induction therapy x 2 weeks with Amphotericin B ± flucytosine.

↓ followed by

Oral fluconazole therapy till $CD_4$ T-cell count >200/microlitre for at least 6 months

# INTRODUCTION TO VIROLOGY

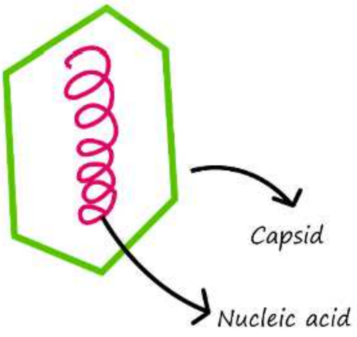
## Viruses 00:01:28

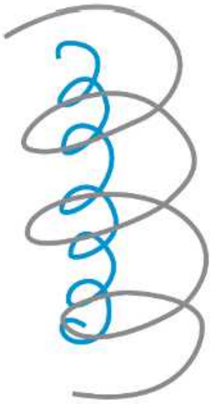
- They are non-living
- They are not cells
- They are smallest Infectious particles
- They are obligate Intracellular
- They have either DNA or RNA, never both.
- They have no ribosomes.
- Viruses are made up of – Protein coat "capsid"
  - Nucleic acid "DNA/RNA"
- Capsid is made up of subunits called as Capsomeres
- **Arena viruses have non-functional ribosomes**

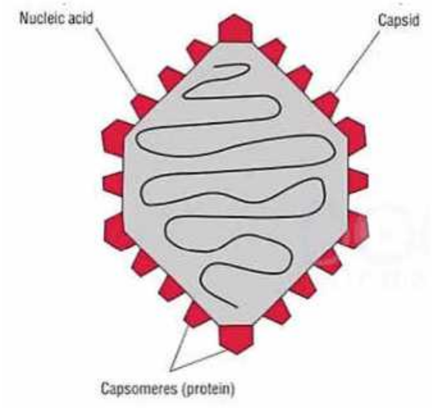
## Symmetry of Viruses 00:05:58

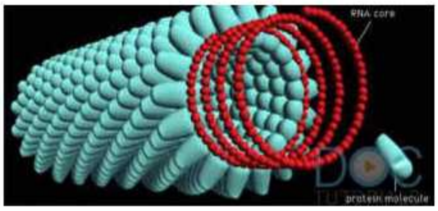
Symmetry of viruses

- Isosahedral symmetry
- Helical symmetry






Nucleic acid
Capsid
Capsomeres (protein)


RNA core
protein molecule

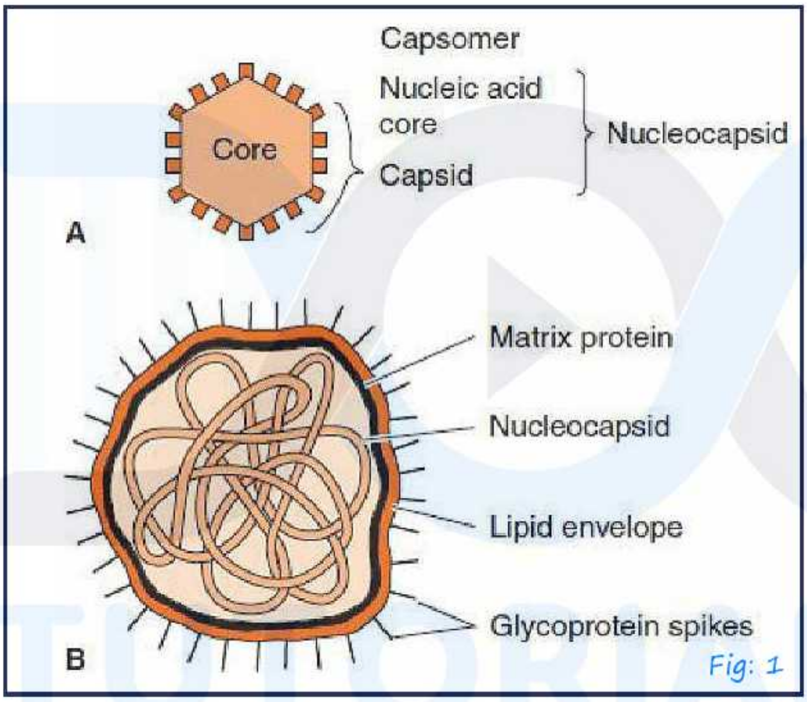

Capsomer
Nucleic acid core
Core
Capsid
Nucleocapsid
A
Matrix protein
Nucleocapsid
Lipid envelope
Glycoprotein spikes
B

Fig: 1

- Envelope: Is an optional structure, it is not present in all viruses.
  - It is made up of phospholipid bilayer & proteins called as peplomers
  - Phospholipid bilayer is derived from host cell membrane
- All Helical viruses are enveloped
- All DNA viruses have Icosahedral symmetry

- DNA virus
  - Env +
  - Env – (Naked viruses)
- Virus attaches to a specific receptor on host cells (cell tropism/tissue tropism)

  Eg: for HIV: $CD_4$ Receptor (present on T-cells & macrophages)

  for EBV: CD 21 Receptor (present on B – cells)
- Virus attaches to host cell receptor with the help of its own proteins:
  - Capsid proteins in naked viruses
  - Peplomers in case of enveloped viruses
- Enveloped viruses are sensitive to ether, alcohol, bile salts
- Enveloped viruses are much easier to kill than non-enveloped viruses
- Once envelope is lost, virus loses its infectivity
- Most viruses which cause infection of GIT – are non enveloped

  Exception: Corona viruses
- Tegument: A structure sometimes present between envelope & Nucleocapsid

## Multiplication of viruses 00:21:46

- Viruses multiply by viral infective cycle

1. Adsorption/Attachment
2. Internalization

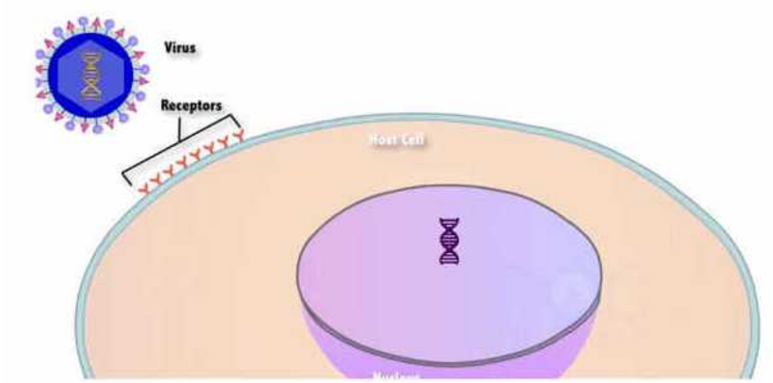
| **Naked** | **Enveloped** |
|---|---|
| • Nucleocapsid/whole virus enters cell | • Envelope stays outside, only nucleocapsid enters host cell. |

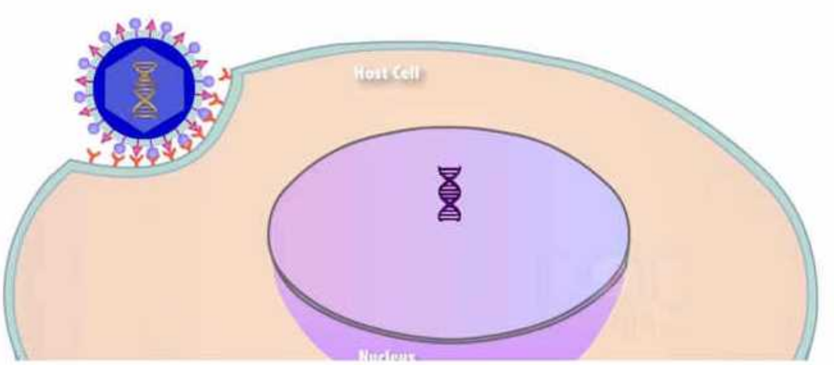
3. Uncoating:

- Capsid is broken down by host cellular enzymes & Nucleic acid

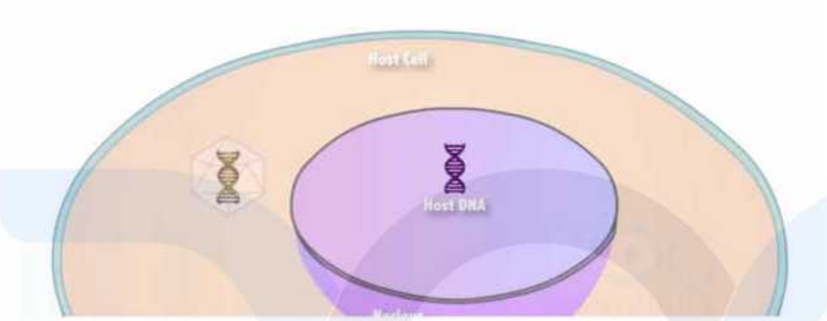
∴ Lies freely in the cytoplasm

4. Biosynthesis
5. Assembly
6. Release – By budding in enveloped viruses
   - By cell lysis in non-enveloped viruses

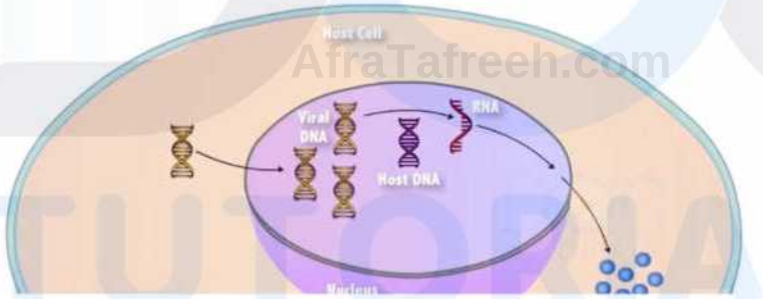



Host Cell
Nucleus

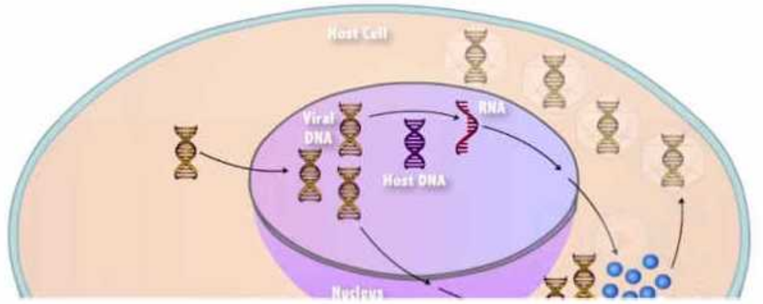
Attachment


Host Cell
Host DNA
Nucleus

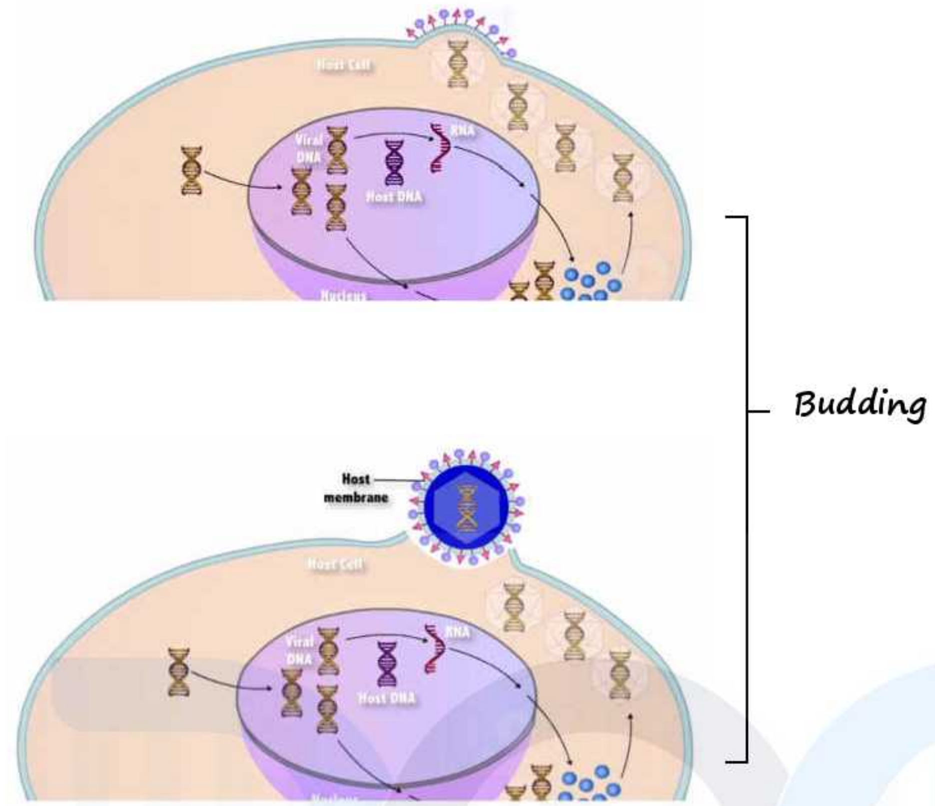
Nucleocapsid entered the host cell


Host Cell
Viral DNA
RNA
Host DNA
Nucleus

Uncoating & Biosynthesis


Host Cell
Viral DNA
RNA
Host DNA
Nucleus

Assembly


Host Cell
Viral DNA
RNA
Host DNA
Nucleus
Host membrane
Host Cell
Viral DNA
RNA
Host DNA
Nucleus
Budding

## Growth/Cultivation of viruses 00:33:10

i. Animal Inoculation – Disease or death

Eg: Coxsackie viruses
&
Arboviruses
– Grow in suckling mice

ii. Growth/Cultivation in fertilised or embryonated hen's egg:

a. Chorioallantoic membrane (CAM):

Pox viruses

Herpes viruses (some of them)

- These viruses form visible lesions on CAM known as POCKS

b. Amniotic cavity: smaller

- Used for primary isolation of Influenza virus

c. Allantoic cavity: larger
  - Used for subculture of Influenza viruses (for vaccines)

d. Yolk sac:
  - Some arboviruses can grow
  - Bacteria: Chlamydiae & Rickettsiae

iii. Tissue culture/Cell culture: most common method to grow viruses. Cell lines are:

a. Primary cell line:
  - In this, cells can multiply upto 5 times

  Eg: Human Amnion Cell culture

b. Diploid cell line:
  - Cells can multiply upto 50 times

  Eg: WI-38

c. Continuous cell line:
  - They are derived from cancer cells
  - They multiply indefinitely

Eg: i. Hela (from human Ca-Cervix)
ii. Hep 2 cell line
iii. Kb cell line

## Methods to detect the presence of viruses in cell lines: 00:42:36

- Cytopathic Effect (CPE)
- Haemadsorption

- Interference
- Metabolic inhibition
- Transformation
- Immunofluorescence – Best method ∵ it is most sensitive & specific

## Cytopathic Effect (CPE):

- It refers to pathological effect on the cell ∵ of the presence of the virus
- Such viruses are known as cytopathogenic viruses
- Viruses which do not cause CPE are known as non-cytopathogenic viruses

## Egs of CPE: 00:45:45

### 1. Inclusion bodies (IBs)

- IBs are viral particles present in infected cell

2 Types

a. Intranuclear IBs

- Produced by DNA viruses (They multiply in nucleus)
- Except Pox virus, which produce intracytoplasmic IBs

b. Intracytoplasmic IBs:

- Produced by RNA viruses, (∵ they multiply in cytoplasm)

  - Except yellow fever virus, which produces Torres bodies – which are intranuclear inclusion bodies
- Measles virus.
  - Produces both intranuclear & Intracytoplasmic IBs. Cytomegalovirus: DNA virus. Produces both – Intranuclear & Intracytoplasmic IBs

### II. Syncytium/Multinucleated giant cell (MNG cell):

- In paramyxoviruses & some herpes viruses (HSV – 1,2, VZV

### III. Grape like cluster formation eg: Adneoviruses

### IV. Shrinking & Degeneration of cells:

- In case of Picornaviruses.

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## DNA viruses 00:55:33

Env+ Herpes viruses
Env+ Hepadna virus /Hepatitis B virus
Adenovirus
Parvovirus – B-19: smallest DNA virus
Env+ Pox Virus – Biggest DNA virus

Papova viruses
- Polyoma viruses – Eg. JC virus
- Papilloma viruses

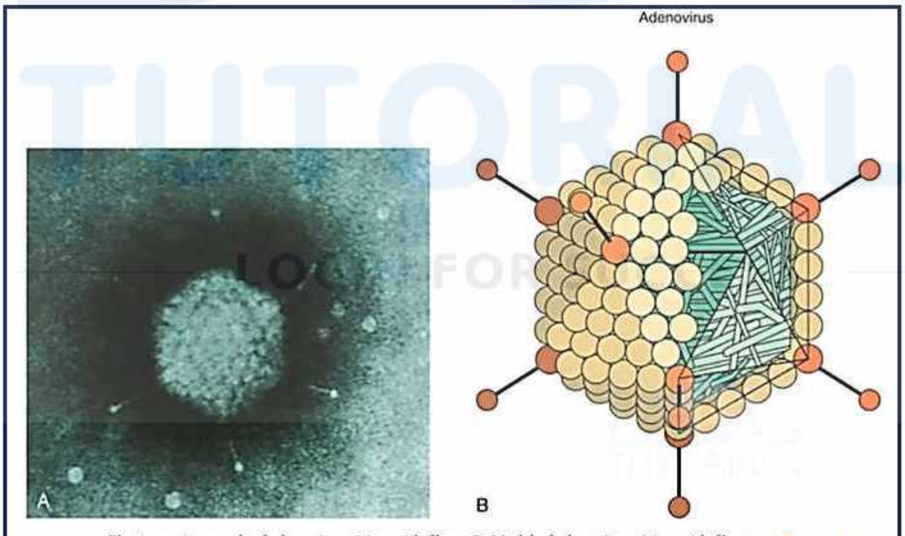
Eg: Human Papilloma Viruses (HPV)

- All DNA viruses have dsDNA except parvovirus B-19 which has ssDNA
- All DNA viruses has Icosahedral symmetry
- No segmented genome in DNA viruses

# ADENOVIRUS

## Adenovirus 00:00:14

- Non enveloped/naked virus.
- Only virus which has fibres arising from is surface: known as Haemagglutinins
- Haemagglutinins – helps in attachment of virus to host cells
- DNA virus
- Icosahedral symmetry
- 47 serotypes
- Adenovirus is also known as "space-satellite-like" virus



Electron micrograph of adenovirus virion with fibers. B, Model of adenovirus virion with fibers. Fig: 1

## Diseases caused by different serotypes of Adenovirus 00:03:40

| Disease | Serotype |
|---|---|
| 1. Pharyngo-conjunctival fever:<br>- A childhood disease<br>- Pharyngitis, fever<br>- There is granular appearance of the bulbar and the palpebral conjunctiva | 3,7,14 |
| 2. Epidemic keratoconjunctivitis or "shipyard eye"<br>- Can be prevented by proper hand hygiene | 8,19,37 |
| 3. Diarrhea<br>- Watery, Non-bloody<br>- In kids < 2 years of age | 40,41 |
| 4. Hemorrhagic cystitis | 11,21 |
| 5. Acute Respiratory Disease | 3,4,7 |

- Adenoviruses also cause neoplasms (not in humans): sarcoma in infected lab rodents (Hamsters) occurs by serotypes 12,18,31

### Diagnosis:

1.Antigen detection by – ELISA,
   - Immunofluorescence

2.Ab - Detection by – ELISA, IIF

3.Culture in – Hela, Hep2 cell lines

# HERPES VIRUSES PART I

## Herpes viruses 00:00:13

- DNA viruses
- 2nd largest after Pox viruses: 120-220nm
- Dictum "Once infected always infected"
- Viruses causes latent infection
- 1st time infection: Known as primary infection

↓

virus lies latent in the body– Irrespective of recovery

↓

Later, reactivates, causing reactivation infection

- **Primary infection:** More severe, associated with more viral shedding
- **Reactivation infection:** Less severe, associated with lesser viral shedding

## Names of Herpes viruses 00:05:38

- Human herpes virus-1: HSV-1
- Human herpes virus-2: HSV-2
- Human herpes virus-3: Varicella Zoster (HZV)
- Human herpes virus-4: Epstein Barr Virus (EBV)
- Human herpes virus-5: Cytomegalovirus (CMV)
- Human herpes virus-6: ⎤ Both causes Roseola
- Human herpes virus-7: ⎦ infantum (or) 6th disease
- Human herpes virus-8: Kaposi sarcoma associated virus

- HSV-1
- HSV-2 ⎬ Cause vesicular lesions
- VZV

- These vesicular lesions when subjected to tzanck smear shows – Multinucleated Giant cells with Intranuclear inclusion bodies

---

## HSV-1 00:08:44

- It causes lesions above the waist
- Mode of infection
  a. Oropharyngeal contact with infected saliva
  b. Direct skin contact

Diseases:

1. Most common infection – Ulcerative Gingivostomatitis
   - On recovery virus becomes latent in trigeminal ganglion
   - Virus which is latent in trigeminal ganglion can reactivate later by any triggering factor like fever, sunlight, stress, menstruation
   - After reactivation – Herpes labialis/fever blisters/cold sores
2. Herpetic keratitis
3. Herpetic whitlow: On the skin of fingers of nurses & doctors

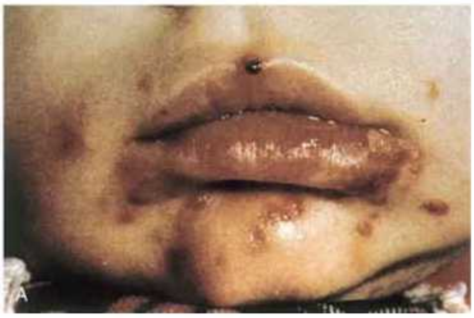


Figure: Herpes labialis Fig: 1

4. Herpetic gladiatorum – In wrestlers
5. Eczema herpeticum – In people with eczema
6. Herpetic encephalitis – Only treatable encephalitis affects temporal lobes
   - Treatment: Acyclovir

HSV-1: is the most common cause of sporadic encephalitis worldwide

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## HSV-2 00:14:57

- Causes infection below the waist
- Mode of infection
  i. Sexual intercourse
  ii. Perinatal – during passage through infected birth canal
- Genital herpes (Below waist):

Painful vesicular lesions in genital region

+

Painful inguinal lymphadenopathy

+

Fever, malaise etc.

- After recovery, virus becomes latent in lumbar and sacral ganglia and can reactivate causing "Recurrent Genital Herpes"
- Vesicular lesions can break down to form ulcers
- Baby can acquire infection while passage through infected birth canal – Neonatal herpes

## Neonatal herpes:

- May be:

  a. Asymptomatic

  b. Mild disease of skin, eyes & mouth

  c. Disseminated disease
- HSV-2: Most common cause of genital ulcer disease in the West
- Genital herpes: 90-95% cases are due to HSV-2 – 5-10% by HSV-1
- Mollaret's meningitis: caused by HSV-2 (Because of retrograde transfer of virus to spinal cord)

**Both HSV-1 & 2 can cause erythema multiforme**

## Diagnosis:

- Diagnosis of both HSV-1 and HSV-2 can be done by:

  Tzanck smear – shows MNG cells with intranuclear inclusion bodies
- Diagnosis of HSV-1 encephalitis is by PCR of CSF

## Treatment:

- Acyclovir
- For HSV keratitis – Trifluridine (Topically applied)

  NOTE:

  a. Both HSV-1 & HSV-2 multiply rapidly (16-18hours) and spread rapidly

  b. Both causes lysis of cells (lytic lesions)

  c. Invade local nerve endings; then travel by retrograde axonal flow & then lie dormant/latent in dorsal root ganglion

# HERPES VIRUSES PART II

## Human Herpes viruse-3 00:00:25

- Also known as Varicella Zoster Virus (VZV)
- 2 diseases:

2 diseases

- Varicella/chicken pox
- Zoster/herpes zoster/shingles

- Mode of infection: Droplet infection
  - Contact with skin lesions

## Varicella 00:02:20

- It is a manifestation of primary infection
- It is a childhood disease-self limiting
- In adults it can be a severe disease
- Treatment in adults: Acyclovir x 5days

**Incubation period:** 14-21 days.

It is followed by a prodrome of fever & malaise

↓

Then maculopapular rash occurs in crops on the trunk, then spreads to head & extremities

↓

- **Rash:** Macules → Papules → Vesicles → Pustules → Scabs

  (Pleomorphic rash/rash in crops)
- After recovery, virus becomes latent in dorsal root ganglia ($T_3$ to $L_3$)
- In adults – Rash can be severe (Hemorrhagic or necrotic too)
- In adults, there are increased chances of complications

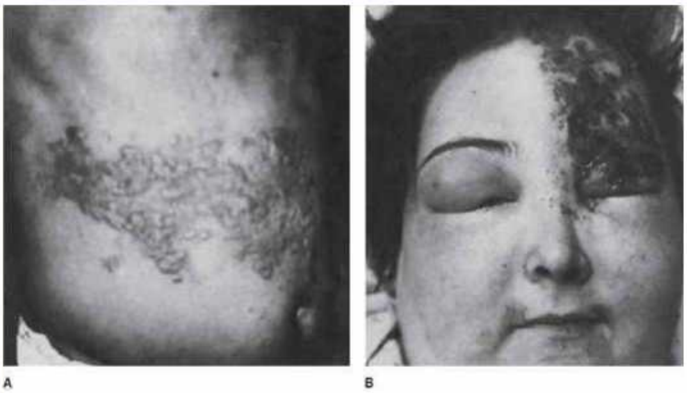
### Complications:

- Most common complication is: Cutaneous

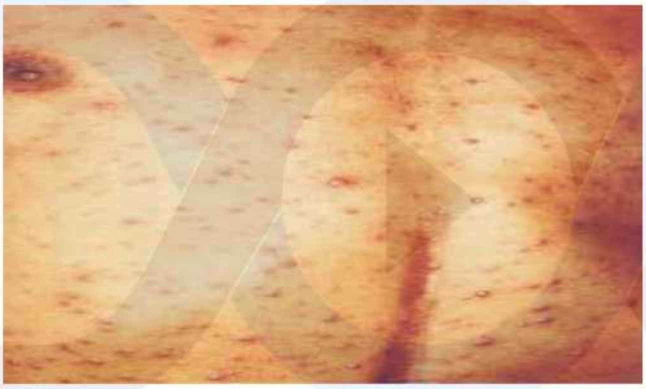
  (Secondary bacterial infection of skin lesions)
- Most common extracutaneous complication is encephalitis
- Most severe complication is pneumonia

## Zoster 00:09:56

- It is a manifestation of reactivation infection
- It is common in 6th decade of life
- There are vesicular eruptions along the course of sensory nerve of head or trunk
- Very painful lesions
- Pain persists even after lesions resolve – post herpetic neuralgia -can be very debilitating
- Does not cross midline



Herpes zoster in the distribution of thoracic nerve and herpes zoster ophthalmicus Fig: 1



Rash of varicella Fig: 2

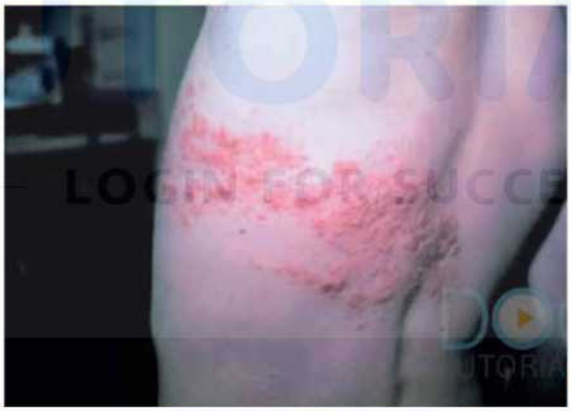


Fig: 3

Zoster (shingles)—note vesicles along the dermatome of a thoracic nerve caused by varicella-zoster virus (figure 3)

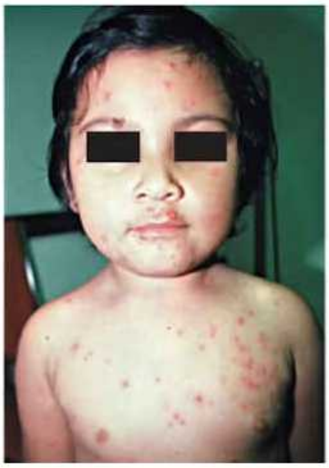


Fig: 4

Varicella (chickenpox)—note vesicles on an erythematous base caused by varicella-zoster virus

- VZV: Can cause "Reye's syndrome"
- Reye's syndrome – Degeneration of liver with hepatic encephalopathy
- Treatment: Acyclovir
- Diagnosis: Tzanck smear shows multinucleated Giant cells with intranuclear inclusion bodies

# HERPES VIRUSES PART III

## Epstein Barr Virus (EBV) 00:00:11

- B-cells are infected by this virus because of CD21 receptors on B-cells
- These infected B-cells undergo malignant transformation – have viral genome
- Peripheral blood smear: atypical lymphocytes
- Atypical lymphocytes:
  - These are not the infected B-cells
  - These are Tc-cells which are reacting against the virus infected cells
- EBV- is ubiquitous
- Modes of transmission:
  - Close contact
  - Sexual intercourse
  - Kissing
  - Blood transfusion
  - Bone marrow transplantation
- The virus lies latent in 'B-cells'
- Initially the virus infects the cells of oropharynx and parotid gland
- Subsequently it infects B-cells (lies latent there)

## Clinical features:

- Most infections are asymptomatic
- In some cases – it causes Infectious mononucleosis/IMN/glandular fever
- It is:
  - A self-limiting illness
  - I.P: 4-8 weeks
  - Lasts for 2-4 weeks
  - Clinical Features:

| | | |
|---|---|---|
| Fever | Splenomegaly | Hepatitis (Subclinical) |
| Sore throat | Arthralgia | Encephalitis (rare) |
| Lymphadenopathy | Lethargy | Rash- usually occurs after patient takes ampicillin |

Rupture of enlarged spleen is a rare but feared complication

- Peripheral blood smear: atypical cells
  - Serum of many patients is positive for heterophile antibodies
- Infectious mononucleosis is also known as kissing disease (common in adolescents & young adults)

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## IMN-like syndrome 00:09:27

- Fever, sore throat, myalgia (No lymphadenopathy)
- IMN-like syndrome is heterophile-negative
- Infectious causes of IMN-like syndrome:

  CMV infection

  Adeno virus

  Toxoplasma gondii infection

  HIV

  Human herpes virus-6

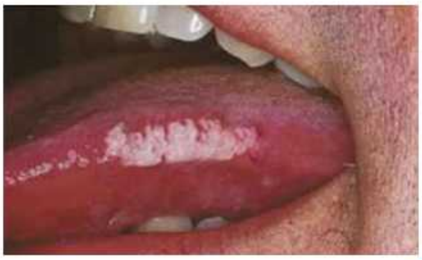
  Ehrlichia
- Non-infectious causes are:
  - Malignancy
  - Autoimmune disease

## Other conditions associated with EBV infection 00:12:44

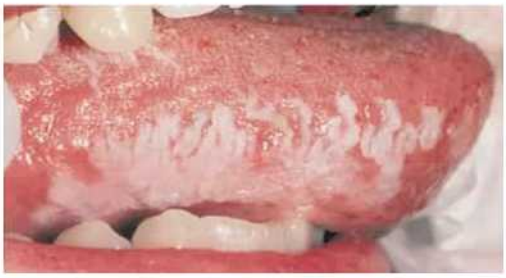
- Burkitt's lymphoma
- Oral hairy leukoplakia
- Nasopharyngeal carcinoma
- Post-Transplant Lymphoproliferative Disorder (PTLD)
- Hodgkin's lymphoma (mixed cellularity type)
- Duncan's disease (X-linked lymphoproliferative disorder)

### Oral hairy leukoplakia:

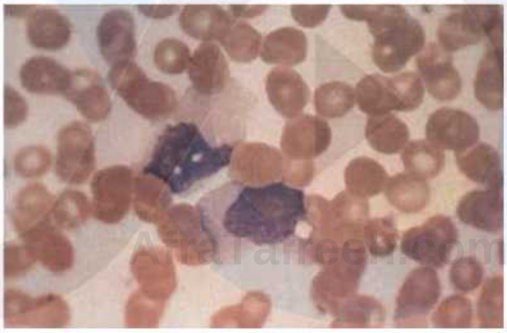
- In HIV patients
- On lateral border of tongue
- Whitish lesion with a rough surface
- Not a premalignant condition



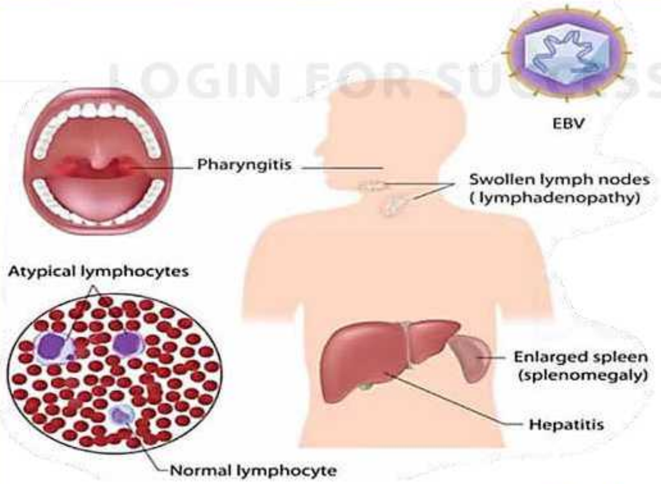
Oral hairy leukoplakia Fig: 1



Oral hairy leukoplakia Fig: 2



Atypical lymphocytes in PBS Fig: 3


EBV
Pharyngitis
Swollen lymph nodes
( lymphadenopathy)
Atypical lymphocytes
Enlarged spleen
(splenomegaly)
Hepatitis
Normal lymphocyte

IMN Features Fig: 4

## Nasopharyngeal carcinoma:

- In Chinese males

- HLA associated: HLA-$A_2$ and HLA-$A_8$

## Post-Transplant Lymphoproliferative Disorder (PTLD):

- It can occur after both bone marrow transplantation & solid organ transplantation
- Most common form is a primary CNS lymphoma (usually a B-cell lymphoma which is EBV associated)

## Duncan's disease:

- Highly fatal form of IMN
- Occurs because of mutation in the gene which encodes for a signal transduction protein needed for the normal activity of NK cells and $T_C$ cells

## Diagnosis of EBV infection:

- Specimen: Blood, saliva, lymphoid tissue
- Culture/isolation: Not done, since it is tough and takes 6-8weeks
- Serology is the mainstay of diagnosis:
  - EBV-specific Abs
  - Heterophile Abs

a. **EBV-specific Abs**

- IgM Ab against viral capsid Ag
- 4-fold or more increase in IgG titre of Abs specific to EBV
- Abs against Early Antigen (EA) seen in

  1.Current infection/early infection

2. Burkitt's lymphoma
3. Nasopharyngeal carcinoma

- Abs against nuclear Ag appear late and indicate past infection – EB Nuclear Ag (EBNA)

**b. Heterophile Abs**

- Detected by heterophile agglutination test: "Paul Bunnel Test"
- In this test, doubling dilutions of inactivated serum of patient (inactivated by heating at 56°c for 30min) is mixed with sheep RBCs. A titre of 1:256 is suggestive of IMN
- Results of Paul Bunnel test are confirmed by Agglutinin Adsorption test:

| Features | Paul-Bunnell test (performed first) | Treated with | | Paul-Bunnell test (repeated) |
|---|---|---|---|---|
| | | Guinea pig kidney cells | Ox red blood cells | |
| Infectious mononucleosis | Positive | Not adsorbed | Adsorbed | • Serum prior treated with guinea pig kidney cells – test is positive<br>• Serum prior treated with ox red cells –Negative |
| Antibody after serum therapy | Positive | Adsorbed | Adsorbed | Negative for both sera |

| | | | | |
|---|---|---|---|---|
| Normal serum | Positive | Adsorbed | Not adsorbed | • Serum prior treated with ox red cells -test is positive<br>• Serum prior treated with guinea pig kidney cells-test is negative |

## Monospot test: A slide agglutination test

- It is a Modified heterophile agglutination test
- Serum which is prior treated with guinea pig kidney and Ox RBCs is mixed with horse RBCs
- This test has – Increased sensitivity (75%)
  - Increased specificity (90%)
- Heterophile Abs – appear early
  - In 40% - in 1st week
  - In 80-90% - by 3rd week
- Heterophile Abs – are negative in old people, kids less than 5years and patients with atypical symptoms
- Most sensitive and specific test for diagnosis of EBV infection – Nucleic acid hybridisation test

## Treatment

- Acyclovir
- In some case: Rituximab

# HERPES VIRUSES PART IV

## Cytomegalovirus/CMV 00:00:22

- CMV causes enlargement of cells which it infects
- It is the largest virus of herpes family
- 1 serotype
- Reservoir is only man
- In vitro: It multiplies only in human fibroblast cell line
- It is also known as salivary gland virus
- Most infections are asymptomatic
- It is the cause of "IMN-like syndrome"
- Important cause of diseases in HIV positive patients & transplant recipients

## Modes of transmission 00:05:05

- It spreads from person to person by close contact
- Transplacental infection – **Before birth**
- Contact with maternal body fluids during delivery – **During Birth**
- By breast feeding – **After birth**
- It also spreads by transplantation of organs
- In kids: Contact with infected urine/saliva
- In adults: By blood transfusion
  - Sexual intercourse
  - Kissing
- Virus multiplies in

  - Salivary glands
  - Respiratory tract cells
  - Kidney
- Virus is shed in
  - Cervical secretions
  - Urine
  - Semen
  - Breast milk
  - Saliva
  - Tears
- Virus remains latent in monocytes
- Virus can persist for years in kidney & shed in urine
- Reactivation of virus in cervical cells can transmit the virus to the baby

## Clinical features:

1. Most infections are asymptomatic
2. Congenital disease – Cytomegalo inclusion disease:
   - Microcephaly
   - Seizures
   - Deafness
   - Petechiae
   - Jaundice
   - Hepatomegaly
   - "Blue Berry Muffin" lesions – Purpuric lesions – occur because of thrombocytopenia

**CMV – Important cause of congenital malformations in West**

- Other features: Chorioretinitis & intracerebral calcification
- These two features – also present in congenital toxoplasmosis

3. IMN – like syndrome (Heterophile negative):
   - Fever
   - Myalgia
   - Lethargy
   - Abnormal LFTs
     - Exudative pharyngitis & cervical lymphadenopathy – absent
4. Infection in Immunocompromised individuals:

Immunocompromised individuals

| AIDs patients | Transplant recipients |
|---|---|
| • Diarrhoea<br>• Retinitis – can cause blindness | • Most common viral pathogen<br>• Infection usually occurs 1-4months after transplant<br>• Manifestations:<br>– Pneumonitis<br>– Esophagitis<br>– Hepatitis |

## Diagnosis:

- Microscopy
  - Intranuclear inclusion bodies – Owl's eye appearance
- Ag-detection:
  - pp65 Ag associated with nucleocapsid by ELSIA
- Ab-Detection
  - By ELISA, IIF, LFT

## Treatment:

- Ganciclovir
- Valganciclovir
- Cidofovir
- Fomivirsen

# HERPES VIRUSES PART V

## Human Herpes Virus – 6 00:00:26

- Tropism for T – Cells
- Binds to CD16 receptor on T-cells
- Has 2-Varaints – 6A, 6B
- Mode of transmission: Infected oral secretions
- In kids: Roseola infantum/6th disease/Exanthem subitem
- Roseola infantum: Characterized by high grade fever and rash
- In adults: mononucleosis like syndrome

## Human Herpes Virus – 7 00:02:14

- 30-50% of DNA homology with HHV-6
- Tropism for T-cells
- Mode of infection: Infected oral secretions
- Disease: Fever, Respiratory and GI symptoms, seizures, Pityriasis rosea

## Human herpes virus – 8 00:04:07

- It is associated with Kaposi sarcoma
- Hence known as Kaposi Sarcoma associated herpes virus (KSHV)
- Infects B-cells
- It belongs to gamma-subfamily of herpes virus

- In high prevalence areas like Africa, kids are usually affected
- Mode of infection: Infected oral secretions
- In low prevalence areas like North America, Asia, Northern Europe – Adults are affected; mode of infection-sexual intercourse; more common in homosexuals

## Associated diseases:

1. Kaposi sarcoma:
    - It is a tumor of endothelial origin
    - Presents with red to maroon lesions on skin, oral mucosa etc. (Figure 1)
    - It is an AIDS defining illness
2. Primary effusion lymphoma:
    - It is a body cavity-based lymphoma
3. Castleman's disease:
    - It is a lymphoproliferative disorder of B-cells

    NOTE: All these three diseases are seen in immune compromised people; In immune competent people → Only fever + Rash is seen

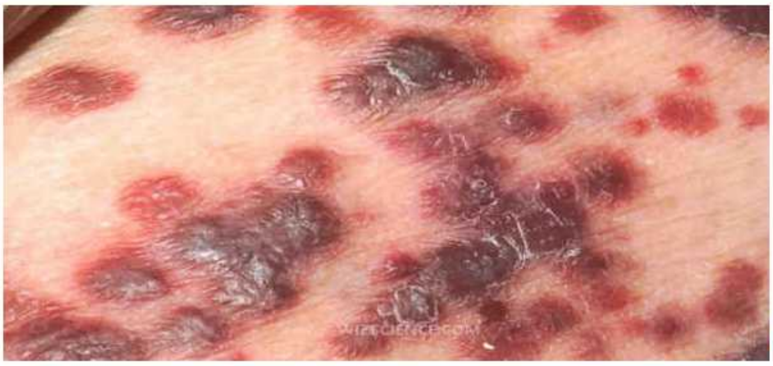


Fig: 1

# HEPATITIS VIRUSES PART I

## Hepatitis viruses 00:00:13

- The primary site of infection is liver
- Hepatitis incidentally can occur in:
  - Marburg virus
  - Lassa fever virus
  - EBV
  - CMV
  - HSV-1 — Herpes Viruses
  - HSV-2
  - VZV
  - Coxsackie viruses
  - Rubella viruses
  - Measles viruses

## Features of Hepatitis viruses 00:03:30

| Features | HAV | HBV | HCV | HDV | HEV |
|---|---|---|---|---|---|
| Family | Picorna viridae (Entero virus 72) | Hepadna virus | Flavi virus | RNA defective virus/no family | Calcivirus |
| Genome | RNA | DNA | RNA | RNA | RNA |
| Envelope | - | + | + | + | - |
| Cultivable | Yes | No | No | No | No |
| Vaccine | Yes | Yes | - | - | Yes (licenced only in CHINA) |
| Modes of infection | Feco-oral route | Blood | Blood | Blood | Feco oral route |

## Hepatitis A-Viruses (HAV) 00:10:41

- Size: 27nm
- Mode of infection: Faeco-oral route
- No chronic/carrier state
- No risk of Hepatocellular Carcinoma (HCC)
- The virus appears in the faeces 2weeks before the onset of symptoms
- Faeco-oral route → From Gut

  ↓

  Blood

  ↓

  Liver

  ↓

  Hepatocytes

  ↓

  Tc cells attack the virus-infected hepatocytes
- Incubation period: 3-4weeks

### Clinical features:

- Fever
- Anorexia
- Nausea
- Vomiting
- Jaundice
  - Increased transaminases
  - Dark urine
  - Pale faeces

- Most cases resolve in 1-3weeks

### Diagnosis:

1. IgM detection by ELISA
   - IgM appears at the time of onset of jaundice
   - Replaced in 2-4weeks by IgG Ab (life long protection)
2. 4-fold or more increase in IgG Ab by ELISA
3. Visualisation of viral particles in faeces
4. Culture of faeces

### Treatment:

- No antivirals
- Vaccine is available
  - Very rarely sexual intercourse (men having sex with men)- HAV infection can occur

↓

Therefore, vaccine is recommended for such people in the West

- Very very rarely: Infection occurs by blood transfusion

## Hepatitis B-Virus (HBV) 00:18:19

- It is the most important cause of hepatitis
- Genome: DNA
- There are 2 linear strands of DNA

i. Complete strand/minus strand

ii. Incomplete strand/plus strand

- The enzyme DNA polymerase is present
- DNA polymerase has 2 activities:
  - DNA dependent DNA polymerase
  - RNA dependent reverse transcriptase
- The genome has genes

1. P
2. C
3. S
4. X

- X gene
  - Gives rise to HBX protein
  - This protein helps in viral transcription & helps in carcinogenesis since it inactivates p53 gene
- S gene
  - Encodes for HBs Ag
  - Referred to as surface/Australia Ag
- C gene
  - Encodes for 'c' Ag & 'e' Ag
- P gene
  - Encodes for polymerase enzyme
- In the serum of a patient of HBV infection, these are 3 types of particles:

a. Spherical particles
  - They are 42nm virions
  - Full virions/DANE particles

- Have the genome of the virus so are infectious

b. Spheres:

- They are 22nm
- No genome – Non infectious

c. Filaments

- They are 22nm
- No genome – Non infectious

- Both spheres & filaments together are referred to as surface Ag/HBs Ag
- Ratio of $\frac{\text{spheres and filaments}}{\text{Dane particles}} = \frac{1000}{1}$

- HBV hepatitis is also known as serum hepatitis
- Antigens of HBV:

i. Common group Ag: 'a'

ii. 2 sets of mutually exclusive epitopes

- i.e., 'd' or 'y' & 'w' or 'r'

| Serotypes | a | d/y | w/r |
|---|---|---|---|
| 1 | adw | 4 serotypes | |
| 2 | adr | | |
| 3 | ayw | | |
| 4 | ayr | | |

- Immunity depends on 'a' Ag

### Modes of infection:

- Transfusion of infected blood and its products

- Sexual intercourse
- Percutaneous (needle stick injury, IV drug abusers)
  - Needle stick injury → 30% risk

    For HIV: Risk is 0.3%

    For HCV: Risk is 3%
- Mother to child transmission: occurs perinatally (during birth)
  - Transplacental transmission is very rare
  - No evidence of transmission of infection during breast feeding
- Mother to child transmission depends on HBe Ag

HBe Ag

- Carrier mothers who are HBe Ag positive
  ↓
  In >90% cases, mother transmits the virus to child
- Carrier mothers who are HBe Ag negative but anti HBe positive
  ↓
  In 10-15% cases mother transmits virus to child

- HBe Ag: Indicates high infectivity of virus, appears in serum.
  - HBc Ag: Core Ag; does not appear in serum

# HEPATITIS VIRUSES PART – II

## Carriers of HBV 00:00:10

- A person who harbors HBs Ag in serum for ≥ 6 months
- Also referred to as chronic HBV infection

Carriers

| Simple carriers ↓ | Super carriers ↓ |
|---|---|
| – HBsAg positive | – HBsAg positive |
| – HBeAg negative | – HBeAg positive |
| – Decreased DNA polymerase | – Increased DNA polymerase |
| | – Increased transaminases |

– With time, many super carriers become simple carriers

## Serology of HBV infection 00:02:50

### A. Antigens

1. HBsAg
   – 1st Serological marker to appear in the serum

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- Appears before the onset of clinical features or increase in transaminases.
- Disappears 1-2 months after the onset of jaundice

  and

  Rarely persists beyond 6 months

2. HBcAg/Core antigen
   - Present on Nucleocapsid
   - Does not appear in the serum
   - In hepatocytes, it can be detected by immune-histochemical staining
3. HBeAg
   - Appears in the serum either concurrently with HBs Ag on shortly after HBs Ag
   - Indicates active intrahepatic viral replication and high infectivity
   - Qualitative marker of viral replication
   - It is measured for following up a patient with HBV infection

**B.Antibodies**

4. Anti-HBc
   - Appears in the serum 1-2 wks after the appearance of Hbs Ag
   - IgM anti HBC – acute infection
   - IgG anti HBC – chronic infection

5.Anti-Hbs

- Protective antibody
- Only marker present in vaccinated people
- Appears in the serum after the disappearance of HBsAg

6.Anti-HBc

- Indicated low infectivity

7. DNA Polymerase

- Quantitative marker of viral replication

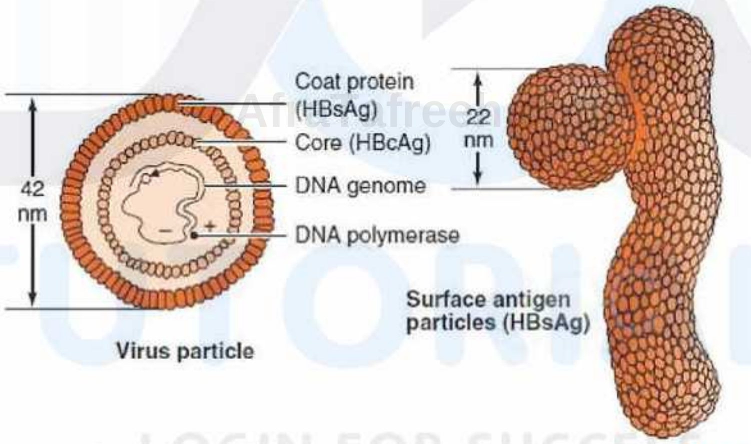

Coat protein (HBsAg)
Core (HBcAg)
DNA genome
DNA polymerase
42 nm
22 nm
Virus particle
Surface antigen particles (HBsAg)

Fig: 1

Fig: 2

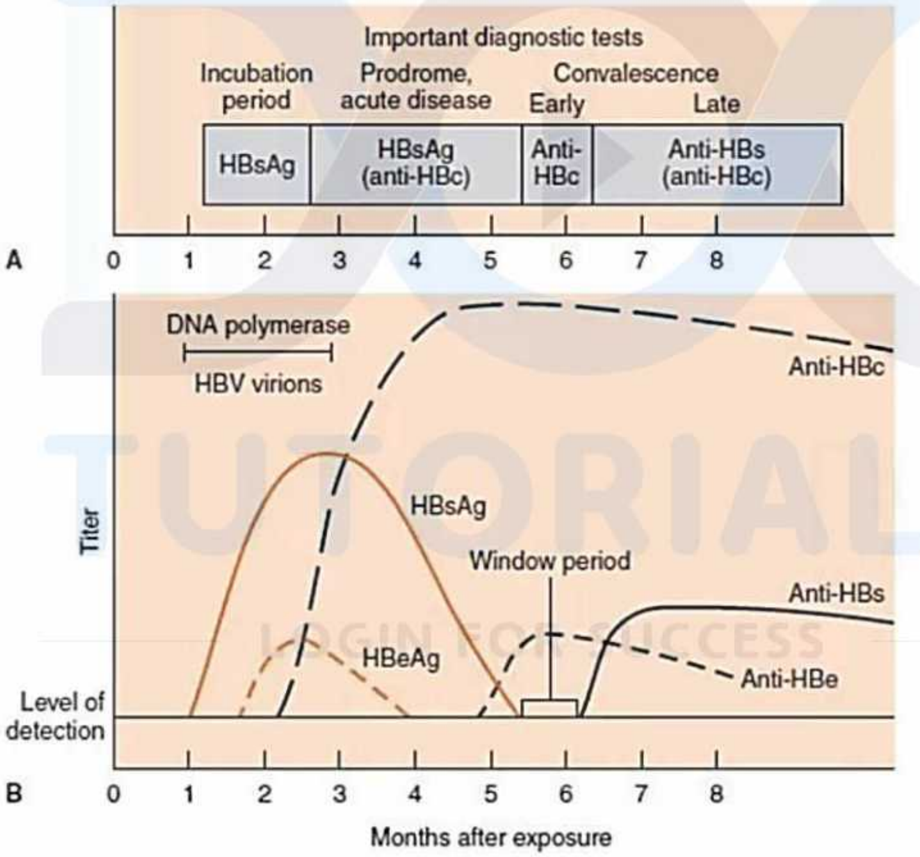
# HEPATITIS VIRUSES PART – III

## Window Period 00:00:14

- Window period is the time period b/w disappearance of HBsAg and appearance of anti – HBs.

### Diagnosis of HBV is done by:

- IgM anti HBC



### Treatment

- No drug is given in acute phase
- In chronic hepatitis: DOC is
  - Entecavir, Tenofovir
  - Peg – IFN – α can be used

- Other drugs – Lamivudine, Adefovir

**Vaccine: Recombinant vaccine:**

[HBsAg is grown in yeast cells via recombinant DNA technology]

- Given to
  1. Healthcare personnel
  2. Travelers going to endemic countries
  3. Intravenous drug abusers
  4. Multiple blood transfusions/dialysis
  5. Frequent STDs

## Hepatitis C virus/HCV 00:06:49

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- Shows antigenic variation
  (gene which encodes for one of the envelope proteins undergoes frequent mutation)
- Flavivirus
- No vaccine
- Causes chronic hepatitis, HCC, Cirrhosis
- Alcoholism increases the risk of HCC because of HCV
- Most infections are asymptomatic
- Even when symptoms occur, they are similar to acute HAV/acute HBV infection but they are milder
- Extrahepatic manifestations:
  Vasculitis
  Arthralgia

Purpura

MPGN (Membrano proliferative glomerulonephritis)

- HCV – Important cause of Cryoglobulinemia

## Diagnosis

- IgM anti HCV (by ELISA)
- Gold standard: RT – PCR (done for RNA viruses )

- 6 genotypes of HCV

## Modes of transmission:

1. Infected blood and blood products
2. Intravenous drug abusers ⎤ Percutaneous
3. Needle stick injury ⎦
4. Sexual intercourse (rare but possible)
5. Mother to child transmission

- Rapid mutation in HCV leads to a no. of subspecies (genetic hypervariable state) – called "quasi-species"

Treatment

In acute case: peg-IFNα

In chronic cases:

Peg. IFN – α

+

Protease inhibitor

+

Ribavirin

### Risk of perinatal transmission of hepatitis viruses:

| HAV | HBV | HCV | HDV | HEV |
|---|---|---|---|---|
| – | +++ | ± | + | – |

## Hepatitis D virus/(HDV) 00:17:43

- No family, defective virus
- Defective virus – because it depends on HBV for its multiplication because it does not have genes for the envelope (figure 1)
- Also called Delta agent

Note – Dane particle – HBV (42nm)

### Modes of infection:

1. Sexual intercourse
2. Blood and blood products transfusion
3. Perinatal transmission

## Coinfection & superinfection 00:20:33

| Coinfection | Superinfection |
|---|---|
| - One simultaneously gets infected with both<br>i. HBV<br>ii. HDV | - A person already infected with HBV gets new infection with HDV |
| - Less severe | - More severe |
| - Decreased chances of developing fulminant hepatitis, chronic disease, HCC. | - Increased chances of developing fulminant hepatitis, chronic disease, HCC. |
| - Dx – IgM anti HBc<br>+<br>IgM anti HDV | - Dx – IgG anti HBc<br>+<br>IgM anti HDV |

**Treatment:**

- Peg IFN – α
  - No vaccine available
  - Vaccination with HBV vaccine confers protection against HDV too.

## Hepatitis E virus/(HEV) 00:24:50

- E for endemic/enteric

  Mode of infection: Faeco-oral route

- No chronic/Carrier state
- No risk of HCC

Clinical features – similar to HAV infection

- Case Fatality Rate (CFR) – 1-2%

  But in pregnant ladies CFR is 10-20%

  - Intense pruritus because of cholestasis

- Dx – IgM anti HEV
  - More prevalent in developing countries
  - No vaccine (except in China)
  - No antivirals

## Hepatitis G virus 00:27:36

- Flavivirus
- But unlike HCV, this virus has not yet been shown to cause liver disease.
- Also known as GB – Virus C
- Modes of infection

  i. Blood and blood product transfusion

  ii. Sexual intercourse

  - 60-70% of infected people clear the virus
- If HIV infected people get infected with this virus:

  ↓ are found to have

  Decreased HIV load (because of decreased HIV multiplication)

  ↓

Because of viral interference

(Hep.G virus interference with multiplication of HIV)

Dx – RT–PCR

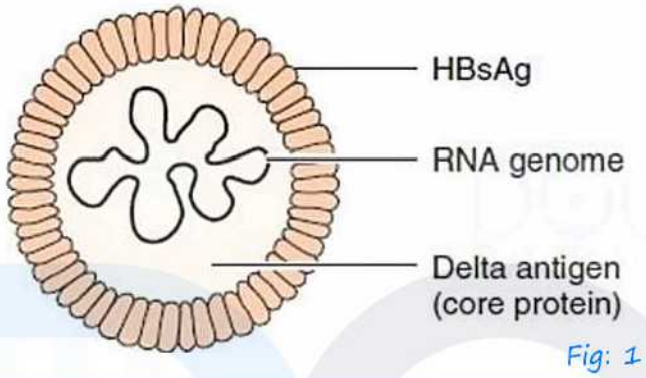

Fig: 1

Note

- Hepatitis – A
  - Most common cause of acute viral hepatitis in children
- Hepatitis – B
  - Most common cause of viral hepatitis
  - Most common cause of acute viral hepatitis
  - Most common viral cause of chronic hepatitis
  - Most common viral cause of HCC
  - Most common cause of Viral hepatitis in pregnancy
  - Most common cause of transfusion – associated hepatitis
  - Most common cause of hepatitis associated with HIV infection.

- Hepatitis C
  - Most common virus associated with chronic hepatitis
  - Most common acute viral hepatitis leading to chronic hepatitis.
  - Most viral cause of cirrhosis
- Hepatitis D
  - Most common virus associated with fulminant hepatitis (superinfection)
- Hepatitis E
  - Most common viral cause of fulminant hepatitis
  - Most common cause of acute viral hepatitis in pregnancy
  - Most common cause of acute viral hepatitis in adults.

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# INTRODUCTION TO RNA VIRUSES

## RNA viruses 00:00:14

- RNA is the genome in RNA viruses
- This RNA is single stranded (ss) except rheoviridae family e.g. Rota virus-Rheoviridae – has Double standard (ds DNA)
- RNA viruses can have
  - Icosahedral
  - Helical symmetry
- All helical viruses are enveloped
- Envelope has proteins known as peplomers
- Peplomers help the virus the attach to the host cell receptors
- Some RNA Viruses have segmented genome e.g. Bunya viruses, Orthomyxoviruses, Reoviruses, Arena viruses
- The viruses with split genome can exchange their RNA segments with related viruses which leads to creation of novel viruses which can cause pandemics/Epidemics
- These novel viruses have antigens different from predecessor viruses and this major change in the structure of Ags is known as antigenic shift
- Such exchange of RNA segments is known as genetic reassortment
- No split genome in case of DNA viruses

## RNA viruses

### Positive (+) sense RNA virus

- RNA itself is xerox copy of mRNA
- Therefore, positive sense RNA can itself be translated into viral proteins & enzymes

### Negative (-) sense RNA virus

- This RNA is not xerox copy instead its complimentary to mRNA
- This negative sense RNA should be changed into positive sense RNA by Viral RNA dependent RNA polymerase
- Positive sense RNA can be translated into viral proteins and enzymes
- This RNA dependent RNA polymerase is absent in positive sense RNA viruses

# MYXOVIRUSES (ORTHOMYXOVIRUS)

## Myxoviruses 00:00:13

2Types

- Orthomyxoviruses
- Paramyxoviruses

Myxoviruses:

- Have affinity for mucin glycoprotein present on: Respiratory epithelial cells & RBCs
- These viruses cause respiratory tract infections

## Envelope spikes of paramyxoviruses 00:08:25

| | H | N | F |
|---|---|---|---|
| Measles virus | + | – | + |
| Mumps virus | + | + | + |
| RSV | – | – | – |
| Para influenza | + | + | + |

- Measles virus & Mumps virus → Fusion protein is haemolytic too
- Mumps & parainfluenza → H,N → Present on same spike & F → Present on a different spike

- Influenza virus: H & N are present on different spikes

## Influenza viruses 00:11:13

- Enveloped viruses
- Genome: Segmented in the form of RNA segments – Negative sense
- Split genome/Segmented genome
- Peplomers are present: Haemagglutinin & Neuraminidase
- Matrix proteins are also present
  - M1 – Present beneath the envelope mediates interaction between envelope proteins & Nucleocapsid proteins
  - M1 protein is responsible for structural integrity
  - M2 – It is an ion channel which connects the viral interior to external milieu
  - M2 protein helps in uncoating of virions in host cell
- In host cell, lysosome has $H^+$ ions/Protons; this lysosome fuses with phagosome
- The $H^+$ ions present in lysosome pass through M2 matrix protein into interior of virus
- This disrupts the envelope of virus because of which the nucleocapsid lies freely in cytoplasm of host cell

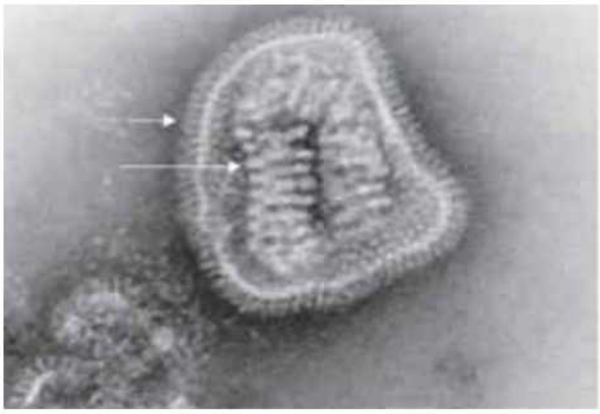

Fig: 1

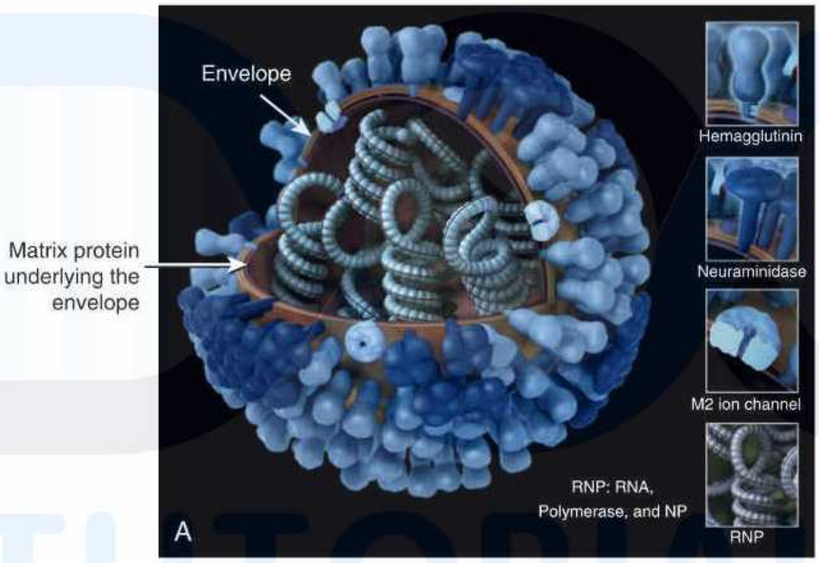
Envelope
Matrix protein underlying the envelope
Hemagglutinin
Neuraminidase
M2 ion channel
RNP: RNA, Polymerase, and NP
RNP
A

Fig: 2

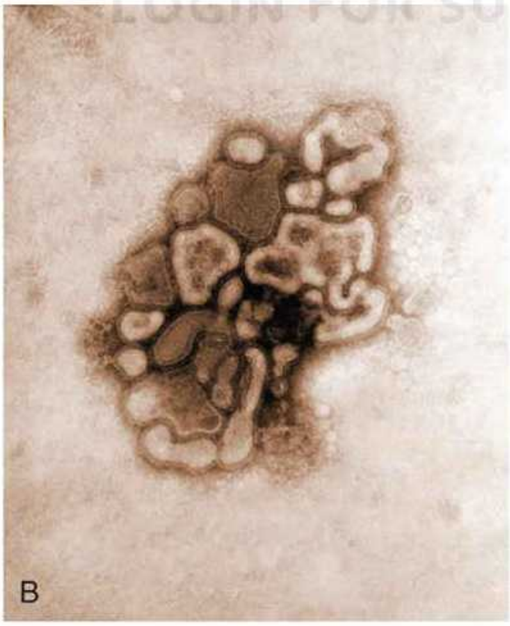

Fig: 3

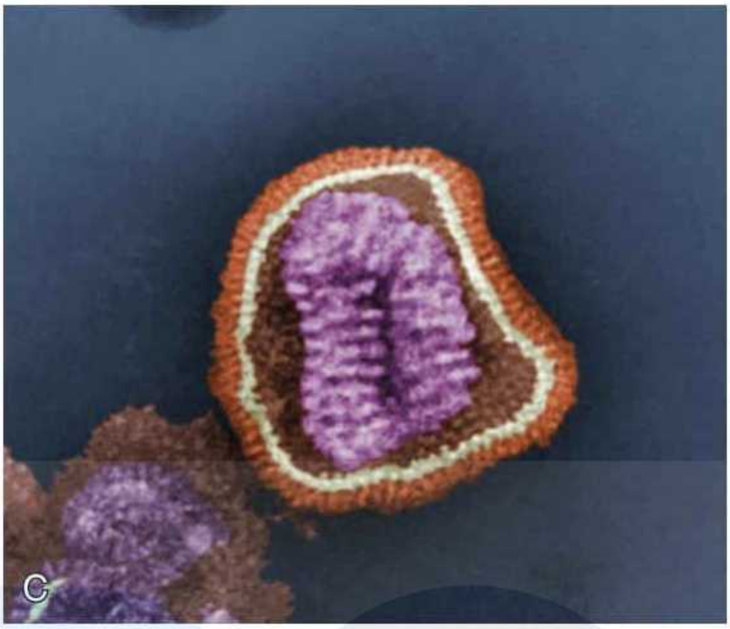

Fig: 4

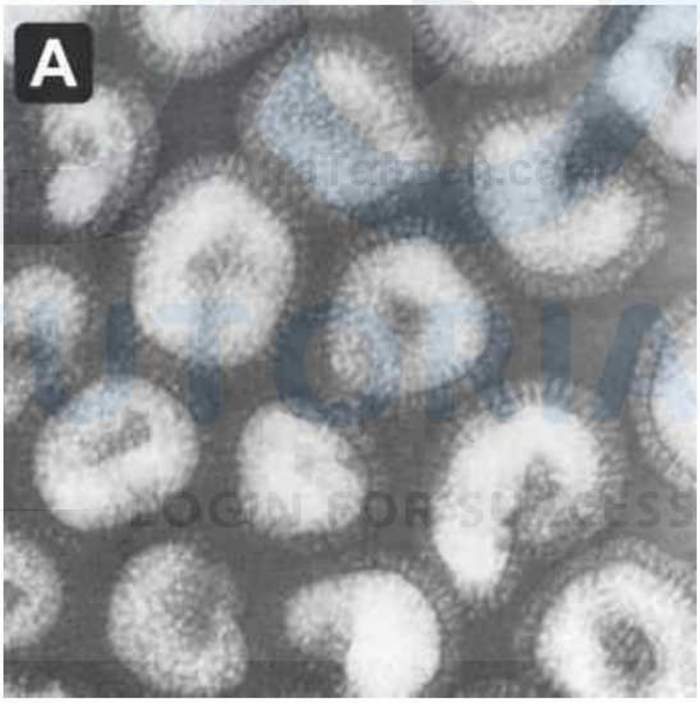

Fig: 5

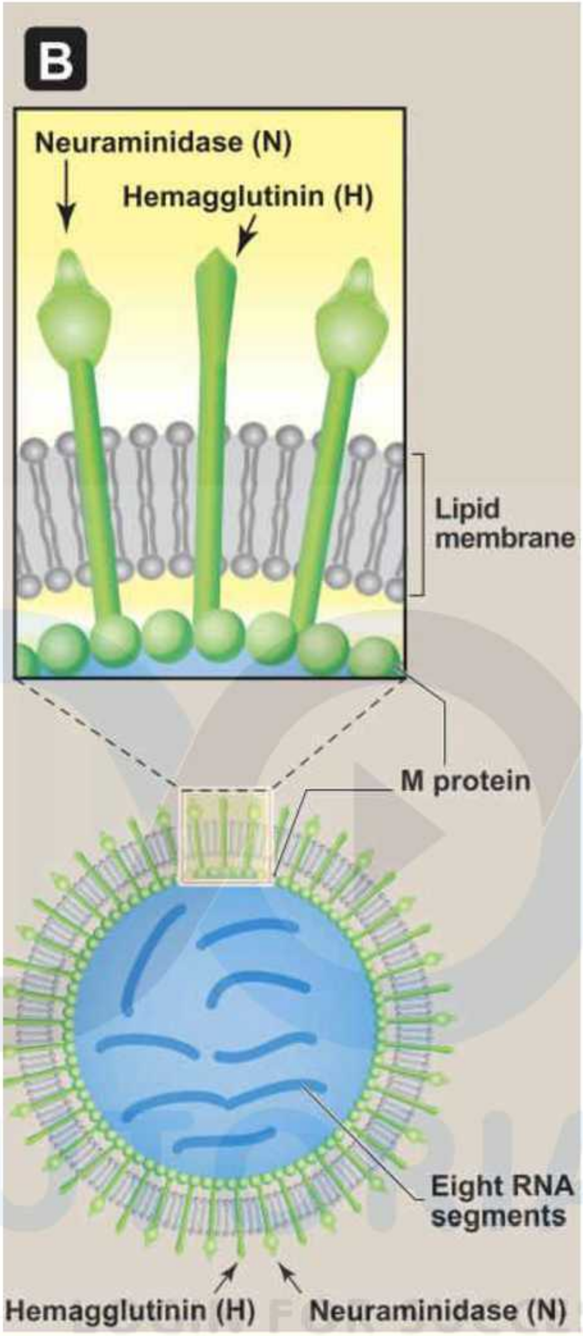
B
Neuraminidase (N)
Hemagglutinin (H)
Lipid membrane
M protein
Eight RNA segments
Hemagglutinin (H)
Neuraminidase (N)

Fig: 6

## Antigen types in Influenza viruses 00:20:30

- Group-specific Ag
- Type-specific Ag

### Group specific Ag:

- They are internal ribonucleioprotein (RNP)
- Does not show antigenic variation

- Based on it there are 3types of Influenza viruses:
  - Influenza A virus
  - Influenza B virus
  - Influenza C virus

## Influenza A virus:

- Has 8 segments of RNA
- Infect humans & animals
- Animal reservoirs are water fowl, chicken, pigs
- This causes major epidemics/Pandemics because of a major antigenic change-Antigenic shift

## Influenza B virus:

- Has 8 segments of RNA
- Infect only humans not animals
- There is no antigenic shift

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## Influenza C virus:

- Has 7 segments of RNA
- Non-pathogenic for humans

NOTE: Each segmented RNA encodes for 1 protein

## Type specific Ag:

- H – Ag
- N – Ag
- They show antigenic variation

## Types of Influenza A virus

(Based on H and N Ags)

- H – Ag
  16 types
- N – Ag
  9 types

- 1 Influenza A virus has 1 out of 16 H types & 1 out of 9 'N' types
  - E.g., Influenza A H1 N1
  - Influenza A H3 N2

## Antigenic variation 00:28:34

- 2 Types of antigenic variation:
  - Antigenic shift
  - Antigenic drift

| Antigenic shift | Antigenic drift |
|---|---|
| • It is a sudden change which occurs because of exchange of RNA segments between Influenza 'A' viruses<br>• This exchange is known as genetic reassortment<br>• It can cause major epidemics/Pandemics<br>• Seen in influenza A virus | • Slow gradual change in H, N structure because of mutation<br>• It does not cause pandemics or major epidemics<br>• Seen in influenza A, B |

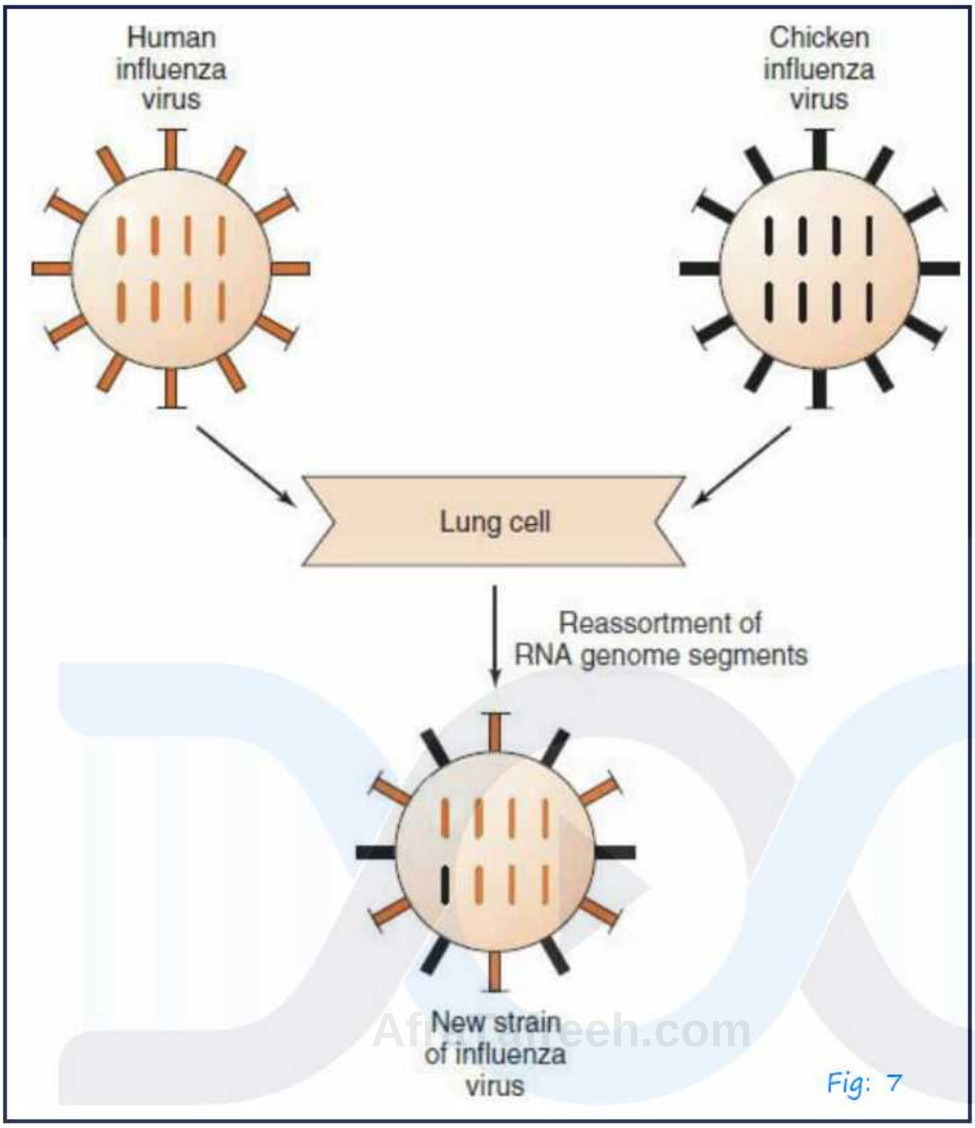
Human influenza virus
Chicken influenza virus
Lung cell
Reassortment of RNA genome segments
New strain of influenza virus

Fig: 7

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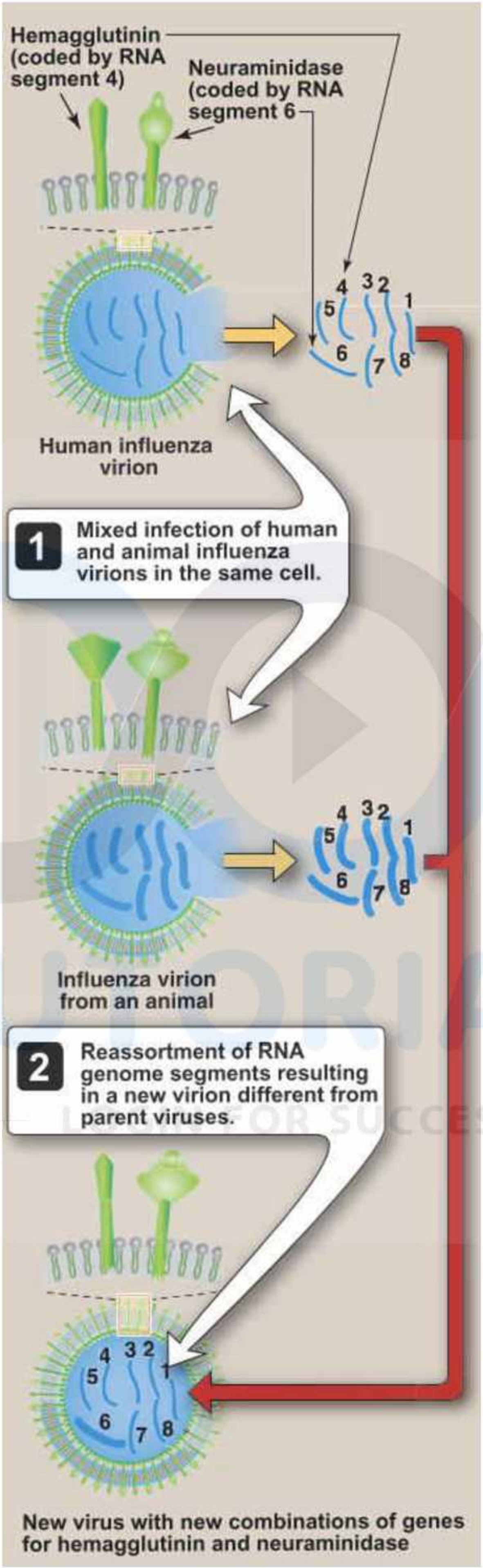
Hemagglutinin (coded by RNA segment 4)
Neuraminidase (coded by RNA segment 6
4
3
2
1
5
6
7
8
Human influenza virion
1
Mixed infection of human and animal influenza virions in the same cell.
4
3
2
1
5
6
7
8
Influenza virion from an animal
2
Reassortment of RNA genome segments resulting in a new virion different from parent viruses.
4
3
2
1
5
6
7
8
New virus with new combinations of genes for hemagglutinin and neuraminidase

Fig: 8

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## Peplomers 00:39:38

- Function of peplomers of the Influenza virus
  - H – Ag (Haemagglutinin)
  - N – Ag (Neuraminidase)

**H - Ag:**

- Plays a role in initiation of infection
- Host proteases cleave 'H' Ag
- The cleaved Ag part attaches to the host cell receptor i.e. Neuraminic acid receptor leading to initiation of infection

**N - Ag:**

- It breaks down the host cell receptor leading to release of daughter/progeny virions which can then attack other cells
- Plays a role towards the end of infection
- Thus, helps in spread of infection
- N – Ag also breaks down the protective mucus layer lining the respiratory tract thereby facilitating the attachment of 'H' Ag to host cell receptors

**Cells infected are:**

- Ciliated respiratory epithelial cells (Most common)
- Mucus gland cells
- Alveolar cells

- Alveolar macrophages/Dust cells
- These cells are infected because of presence of host proteases in these cells

## Disease caused by Influenza viruses 00:47:54

- These cause respiratory tract infection (RTI)
- Modes of infection
  - Inhalation of droplets & small particle aerosols
  - Contact with fomites
- Incubation period: 24 – 48hrs
- Following the IP patient develops:

  Fever, Sore throat, Headache, Myalgia & cough

Running nose – Usually not seen in influenza

- Fever + Myalgia + URT symptoms – Best defines the influenza
- Sometimes bowel symptoms like vomiting & Diarrhoea can occur
- These bowel symptoms mostly caused by influenza – B virus
- Reye's syndrome rarely by Influenza-B virus: Liver degeneration + Encephalopathy
- Reye's syndrome – Also caused by varicella zoster virus
- Complications can occur in elderly & kids
- Complications include:

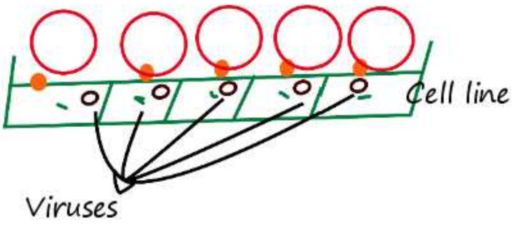
  - 2° bacterial pneumonia By Organisms: S. aureus & Pneumococcus
  - 1° viral pneumonia
- Viremia does not occur but there are systemic symptoms because of release of cytokines
- There occurs necrosis of superficial layer of respiratory epithelium

## Diagnosis:

- Best specimen: (Nasopharyngeal aspirate/swab)
- Others: Nasal aspirate, sputum
- Swab should be kept in viral transport medium at a temperature of 4°C for 4days or at – 70°C for longer periods

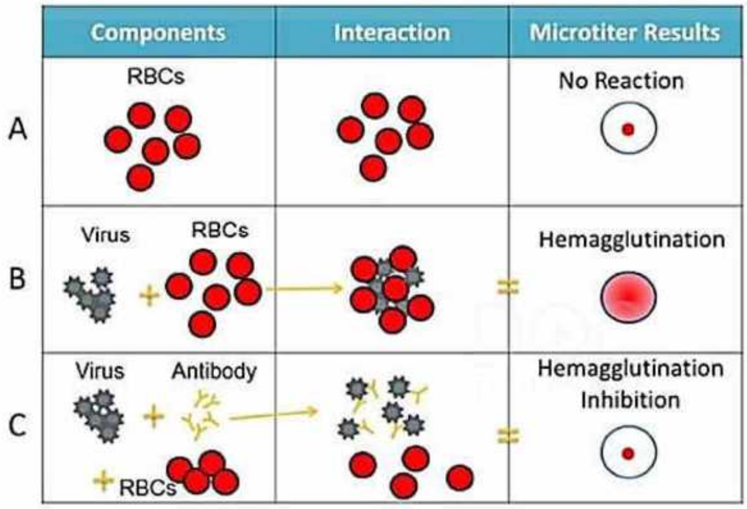
1.Culture/Isolation:

  - Amniotic cavity of fertilized/Embryonated hen's egg can be used
  - Cell lines: primary monkey kidney cell line
  - Growth of virus is detected by hemadsorption or haemagglutination
  - Hemadsorption–it is one of the cytopathic effects



2. Ag detection by direct immunofluorescence (DIF) test
3. RT – PCR: Revolutionary method, turn-around time is ≈ 1day
4. Real time RT – PCR: Gold standard for diagnosis of influenza
   - Diagnosis of influenza
   - It is quantitative, so tells us the viral load
   - Can detect specific type & subtype of influenza virus
   - Result is obtained in 2-3hrs
   - Very good sensitivity & specificity
5. Ab – Detection is serum sample by
   - Haemagglutination inhibition (HAI)
   - Neutralization test: Can detect type specific Abs
   - ELISA– Done most commonly

> Detection of a 4-fold or more increase in Ab titer in period sera taken 10-14days apart is more significant than testing a single serum sample

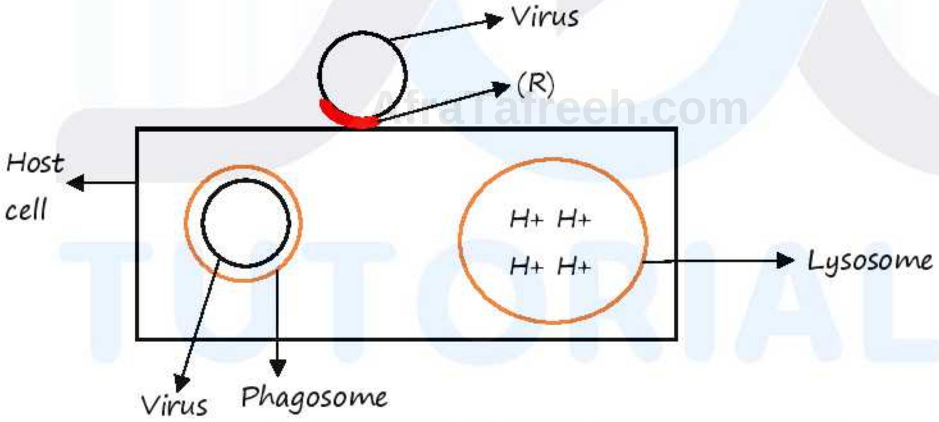

Components
Interaction
Microtiter Results
A
RBCs
No Reaction
B
Virus
RBCs
Hemagglutination
C
Virus
Antibody
RBCs
Hemagglutination Inhibition

**Treatment:**

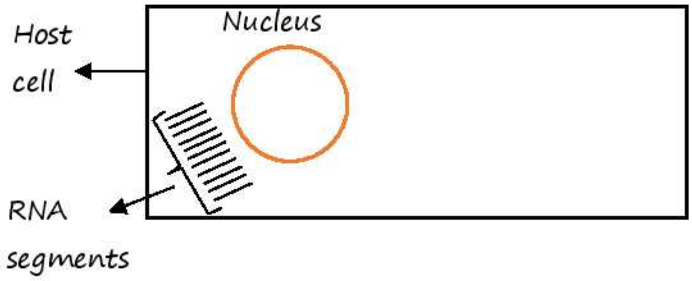
- Oseltamivir (Tamiflu) – Oral drug
- Zanamivir (Relenza) – Inhalation route
- These are used for the treatment both Influenza A & B infections
- These are neuraminidase inhibitors
- Amantadine ⎤ For treatment of influenza A but not Influenza B
- Rimantadine ⎦

## Replication of Influenza viruses 01:13:04

- Even though it is an RNA virus, part of multiplication occurs in nucleus but not in cytoplasm


Virus
(R)
Host cell
H+ H+
H+ H+
Lysosome
Virus
Phagosome

- Phagosome & lysosome fuse and form phagolysosome, $H^+$ enter through M2 and help in uncoating of virus


Nucleus
Host cell
RNA segments

- In nucleus: RNA segments which are of negative sense are converted into positive sense RNA segments

Negative sense RNA segments $\xrightarrow[\text{Polymerase}]{\text{Viral RNA}}$ Positive sense RNA segments

- In host cell nucleus: Host cellular RNA with a methylated guanosine cap
- Some negative sense RNA in the nucleus undergoes replication to form the daughter RNA molecules

# PARAMYXOVIRUSES (MEASLES VIRUS)

## Paramyxoviruses 00:00:28

- RNA viruses
- Negative sense RNA
- Helical nucleocapsid
- Envelope is present with– H, N, F
- Measles virus
  Mumps virus
  Respiratory syncytial virus
  Parainfluenza virus

## Measles virus 00:03:16

- Measles – childhood disease
- Only 1 serotype
- 1 attack gives lifelong immunity
- No chronic/carrier state
- Mode of infection:
  - Inhalation of respiratory droplet (Travel short distances)
  - Inhalation of small aerosols – aerosols remain suspended in air for long in crowded areas like schools
- Incubation period: 10-14 days
- Measles prodrome
  - Fever

  - Cough
  - Coryza
  - Conjunctivitis
  - Photophobia
- Day 10 – fever develops
- Day 12 – koplik's spots develop
- Day 14 – rash develops

- Koplik's spots
  - Pathognomonic of measles
  - They are bright red/ erythematous lesions with a central bluish or whitish dot
  - They first appear opposite lower, second molars; then progresses to the rest of buccal mucosa
- Rash of measles:

  Appears first behind the ears

  ↓

  Face

  ↓

  Spreads from top to bottom, including palms and soles
- Rash disappears in the same order
- It is a maculopapular rash, assumes a brownish hue in a few days – can become confluent too

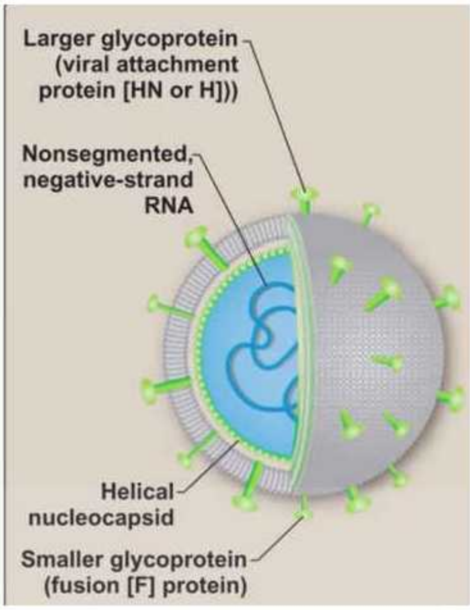

Larger glycoprotein (viral attachment protein [HN or H]))
Nonsegmented, negative-strand RNA
Helical nucleocapsid
Smaller glycoprotein (fusion [F] protein)

Fig: 1

- Warthin – Finkeldy giant cells

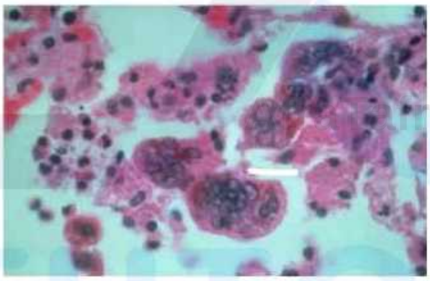


Fig: 2

- Measles rash

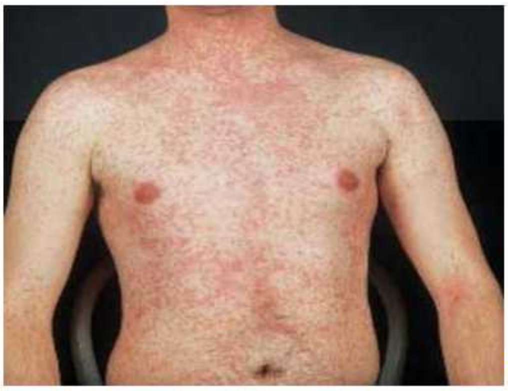


Fig: 3

- Measles rash

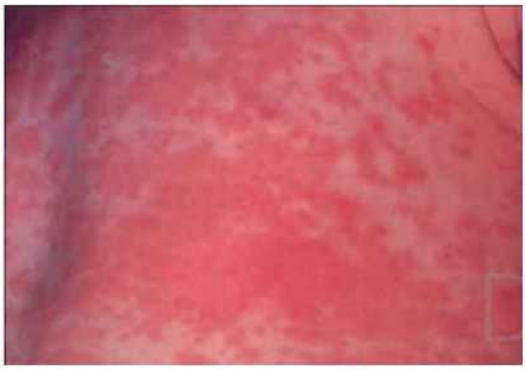


Fig: 4

- Fever and koplik's spots – disappear with onset of rash

Basis of rash:

- Cytotoxic T – cells/Tc cells attack the virus infected endothelial cells
- Ab – mediated vasculitis
- Shortly after the rash appears, the virus can no longer be isolated from the patient and patient cannot transmit the virus to others
- Measles virus infection during pregnancy causes still birth
- Characteristic cytopathogenic effect by measles virus – warthin – Finkeldey giant cells
- Measles virus also known as rubeola virus
- Complications of measles:

  1.Secondary bacterial infections are more common

    - Otitis media
    - Pneumonia

- Decrease in cell mediated immunity: - because measles virus binds to CD 46 receptor on the macrophages, this leads to decreased production of IL-12 which is needed for formation of Th1 subset
- Due to the decrease in CMI, the reactivation of underlying Tb and loss of PPD reaction occurs, i.e., negative Mantoux test

2.Complications due to measles virus itself

- Giant cell pneumonia /Hecht's pneumonia
- Acute LTB/croup
- Diarrhea – which leads to malnutrition including vit -A deficiency

3.CNS complications: Rare but very severe

- Post – measles encephalomyelitis
  - Occurs 2 weeks after an attack of measles
  - It is an auto immune reaction to myelin basic protein (MBP)
- Measles inclusion body encephalitis
- SSPE (Sub acute sclerosing panencephalitis)
  - Caused by defective measles virus (Group – C slow viruses)
  - Seizures, deterioration of cognitive and motor function
  - It usually occurs 7-8 years after measles
  - Becomes fatal within 1-3 years

## Diagnosis:

Sample: Nasopharyngeal swab – best

Others: conjunctural swab

1. Ag – detection by DIF using monoclonal Ab to ribonucleoprotein
2. Culture in primary monkey kidney cells

   CDC recommended cell line is vero/HSLAM

   HSLAM – human signaling lymphocytic activation molecule
3. RT – PCR
4. Serum Ab detection by ELISA, neutralization test, hemagglutination inhibition (HAI) test

   Measles vaccine: strain used is Edmonston-Zagreb strain

   Rash – absent in HIV infected people.

# PARAMYXOVIRUSES

## Mumps virus 00:00:09

- H, N, F Peplomers are present
- H and N– on same spike
- Disease: Mumps, a childhood disease
- Only 1 serotype
- No animal reservoirs
- 1 attack – gives lifelong immunity
- I.P: 14-18 days (avg – 19 days)
- Mode of infection
  - Respiratory droplets
  - Rarely fomites
- No respiratory symptoms
- But multiplies in the respiratory tract and regional LNs

  ↓

  Then spills into blood

  ↓

  Reaches different tissues and organs

### Clinical features:

- Most of the infections (≈ 50%) are asymptomatic
- Most common manifestation (in 70-90%) in symptomatic cases – fever + bilateral parotitis;

bilateral parotitis – pain increases on taking citrus juices.

Rarely, unilateral parotitis occurs

- Other salivary glands sometimes maybe involved like submaxillary and submandibular glands
- Second most common manifestation in 15-30% of post pubertal males: unilateral orchitis
- Mumps (Unilateral orchitis) – does not lead to sterility
- Aseptic meningitis (10% of cases)
  - Self-limiting
  - Male predominance
  - Deafness maybe permanent due to cranial nerve palsy

  0.2% of people may develop encephalitis - fatal
- Oophoritis – in 5% of females
- Pancreatitis
  - In 4% of people
  - Can lead to diabetes
- Rare manifestations:
  - Arthritis
  - Renal dysfunction
  - Myocarditis
- Atypical mumps: Aseptic meningitis without involvement of salivary glands

## Diagnosis:

Sample – Buccal or oral swab

- Ag – detection by direct immunofluorescence
- Culture in primary kidney cells
- RT – PCR
- Serum Ab – detection by ELISA, neutralization tests, Hemagglutination inhibition

## Vaccine strain: Jerryl Lynn strain

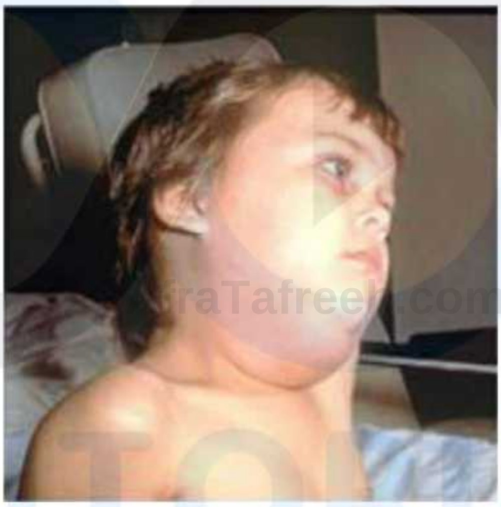


Fig: 1

# PARAMYXOVIRUSES (RESPIRATORY SYNCYTIAL VIRUS, PARAINFLUENZA VIRUSES)

## Respiratory Syncytial Virus (RSV) 00:00:26

- Human respiratory syncytial virus
- Enveloped, RNA viruses – negative sense
- It does not have H or N peplomers
- Only F (Fusion protein) is present

### Mode of infection:

- Large droplet inhalation
- Contact with fomites (carrying virus) and then touching anterior nares, conjunctive and mouth
- It is an important nosocomial pathogen

### Diseases:

i. Most common cause of LRTI in kids

- Bronchiolitis, pneumonia, tracheobronchitis in 25-40% of kids
- Symptoms – fever, rhinorrhea
  - Followed by cough, wheezing, dyspnea

### Chest X– ray of kids:

- Diffuse interstitial infiltrates
- Peribronchial thickening
- Lobar consolidation (occasionally)

- Severe disease in seen in
  - Immunocompromised kids
  - Premature infants
  - Congenital heart diseases
  - Nephrotic syndrome
  - Bronchopulmonary dysplasia
- In adults
  - RSV causes influenza like URT symptoms
  - LRT can be involved in:transplant recipients,
    Immunocompromised adults,
    Elderly people staying in nursing homes
  - RSV can cause exacerbation of asthma and COPD.

## Diagnosis:

Specimen – nasopharyngeal secretions

1. Ag – deletion by ELISA and DIF
2. Culture in Hela and Hep2
   - These are most sensitive cell lines
   - RSV – fragile virus, therefore, specimen should be transported and processed immediately
   - Characteristic cytopathogenic effect of RSV is syncytium formation – appears in 7-10 days
   - Shell vial technique of culture can grow the virus in 1-2 days
3. RT – PCR

## Management

- Palivizumab – monoclonal Ab against F- peplomer
- Ribavirin
- Supportive care
- Ab-detection by ELISA or neutralization test can be done in older kids and adults but Abs are absent in kids < 4 months of age

## Parainfluenza viruses 00:12:25

- 4 types – 1,2,3,4
- Prevalent worldwide
- Prevalent in winter season
- H, N, F peplomers are present
- H and N are on a single spike
- Mode of infection: respiratory droplets
- Disease in kids: Acute laryngotracheobronchitis or croup
  - This leads to narrowing of airway – wheezing/stridor
  - Characteristic "Barking cough" or "seal-like cough'
- In adults: common cold like illness
- Most common complication – otitis media
- This virus infects both humans and animals, but animal viruses do not infect humans

## Diagnosis:

Specimen

- Nasal washes
- BAL
- Lung tissue

1. Ag – detection by DIF
2. Culture: in primary kidney cells
   - No CPE of the virus itself in seen but growth of the virus can be detected by hemadsorption using guinea pig RBCs
   - Ag – Detection using DIF
3. RT - PCR
   - Treatment: symptomatic care

# RUBELLA VIRUS

## Rubella virus 00:00:13

- Disease: Rubella/ German measle s/ three-day measles
- It is not a paramyxovirus
- Belongs to family togairidar but it's not an arthropod borne virus
- Arbovirus
  - Bunyaverider
  - Togairsidar
  - Flaviviridae
- Mode of infection:
  - by respiratory droplets
  - transplacental
  - important cause of congenital malformation
- Icosahedral symmetry
- Enveloped virus
- Has haemagglutinin peplomers
- RNA Genome: positive (+) sense
- I.P: 14-21 days

  ↓

  Virus infects the URT and Regional LNs

  ↓

  Reaches blood and goes to various tissues and causes
- Typical feature: Posterior auricular lymphadenopathy
- Rash:

  - Similar to measles mash
  - Lasts for 3-days
- Basis of rash: Ab – mediated vasculitis
- Arthralgia is caused on adults (F>M)
- 1 attack – gives lifelong immunity
- This is a teratogenic virus

  If pregnant females get infected in first trimester, specially in first month during which organogenesis occurs – congenital rubella syndrome (CRS) occurs

  - CRS: triad of
    a. Heart defects
      - PDA is most common
      - Pulmonary artery stenosis

    b. Eye involvement
      - Cataract (most common)
      - Glaucoma

    c. Sensorineural hearing loss
- Mental retardation also occurs
- Kids infected in utero can continue to excrete the virus after they are borne. This can serve as an important public health hazard for pregnant females

## Diagnosis:

1. Culture – rubella virus does not produce any cytopathic effect but it interferes with CPE of echovirus

- Viral interference: Therefore, specimen when added to cell line

  ↓

  Echovirus is added

  ↓

  No CPE of echovirus is produced

  This is because of presence of Rubella virus infection in specimen.

2.RT -PCR

3.Serum Abs can be detected by Elisa and HAI

- Current infection
  - Ig M abs and 4-fold increase in
  - Ig G abs
- If a pregnant woman has Ig M Abs – Indicates acute or current infection
- But if a titer of ≥ 1:8 of Ig G is present, it indicates past infection
- Vaccine: live attemeuated vaccine – RA 27 B strain
  - Mild side effects (except transient arthralgia)
  - Vaccine is contraindicated in immunocompromised people and pregnant ladies.

# RABIES VIRUS

## Rhabdoviridae family 00:00:14

**Rhabdoviridae family**

Genus lyssa virus
↓ includes
Rabies and related viruses

Genus vesiculo virus
↓ includes
Vesiculo- stomatitis viruses

## Rabies virus 00:01:18

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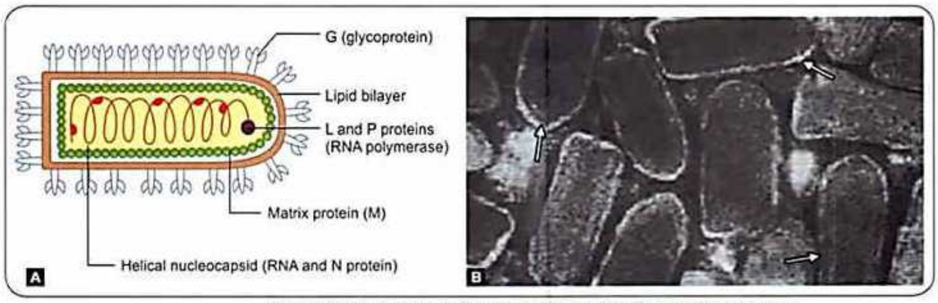
- Causes acute infection disease of CNS (Fatal encephalitis) in humans and animals
- One serotype
- 7 antigenic variants: different animal reservoirs, located in different geographical regions
- RNA virus → negative sense single stranded RNA – genome
- Helical nucleocapsid
- Enveloped virus
- 'E' – has glycoprotein – G spikes → (10nm long)


G (glycoprotein)
Lipid bilayer
L and P proteins (RNA polymerase)
Matrix protein (M)
Helical nucleocapsid (RNA and N protein)
A
B

Rabies virus. A. Schematic diagram; B. Electron micrograph (*arrows showing*)

Fig: 1

- Beneath the envelope is a layer of matrix 'M' proteon

- Bullet shaped – 75nm α180nm
- Glycoprotein G-spikes are significant – because they help in pathogenesis and neuroinvasion

## Rabies virus can infect many warm-blooded animals and humans

- Very highly susceptible animals
  - Wolves
  - Forces
  - Jackals
  - Cotton rats

- Highly susceptible animals
  - Rabbits
  - Cat
  - Cattle
  - Bats
  - Racoons
  - hamsters
- Moderately susceptible animals
  - Dogs
  - Sleep
  - Goat
  - Horses
- Low susceptible animals – opossums
- Mode of infection – bite of a rabid animal

  - ("Human to human transmission is possible but not documented")
- Rare modes of transmission
  - Corneal transplant
  - Aerosol inhalation
  - Licks on cracks of mucous membrane

## Street virus 00:10:29

- Freshly isolated or freshly grown strains of virus in the lab
- Mimics the wild virus
- Producers' intracytoplasmic inclusion bodies
- Long and variable inculcation period
- Multiplies slowly

## Fixed virus 00:11:45

- When the street virus is grown repeatedly in brawn of rabies → fixed virus
- Does not produce intracytoplasmic inclusion bodies
- Does not multiply in extra-neural tissues
- Multiplies rapidly and has shorter inculcation period in 4-6 days
- Used for vaccination

## Pathogenesis

- Rabies has very long and variable Ip (one week to 19 years) average Ip – 20-90 days
- After being bitten by a rabid animal

  ↓

  Initially the virus multiplies locally in muscle and connective tissue

  ↓ then

  Virus enters peripheral motor nerves (by binding nicotinic ach R)

  ↓ then

  Via recto grade axonal transport, it ascends up (250mm/day)

  ↓

  Reaches dorsal root ganglia of spinal cord

  ↓

  Reaches CNs, where it multiplies

  ↓

  Spreads centrifugally via sensory and motor neurons to various tissues and organs of body
- Man titer is present in the salivary glands
  - Pancreas
  - Kidneys
  - Heart
  - Retina
  - Cornea

  Note: no viremia

- Virus is present in high titers in saliva of rabid animals
- In CNS – virus multiplies in cerebellum and hippocampus.

## Histopathological changes in CNS 00:18:51

- Mononuclear cell infiltration
- Perivascular cuffing of lymphocytes
- Babes nodules made of glacial cells
- Negri bodies
  - Intracytoplasmic, capnophilic spherical to oval, 2-10cm inclusion bodies
  - Not seen in 20% of cases
  - Absence of visualization of Negri bodies does not rule out a diagnosis of rabies
  - Most abundant in – purkinjecells of cerebellum and pyramidal cells of hippocampus
- The most site overall of Negri bodies – hippocampus

IP: -

- IP is distancing the virus has to travel from the site of inoculation to CNS
- Therefore, IP is shorter in
  - Short people
  - Kids
  - Bites on head, neck, face, upper limb

- Genetic pre disposition
- Immunocompromised
- Severe lactation
- Viral factor
  - Virulent strains
  - High inoculum

## Clinical features 00:23:50

i. Prodromal phase – 2 to 10 days

- Fever, malaise
- Sore throat
- Anoremia
- Nausea, vomiting
- Abnormal sensation at the site of bite (pairs pruritus, paresthesia)

ii. Acute neurologic phase – 2 to 7 days

A. Encephalitic/furious rabies

1. Phase of hyper-excitability
   - Anxious, imitable
   - Agitated
   - Bizarre behavior
   - Hallucinations
2. Phase of lucid interval/lucidity: -
   - Becomes shorter as the disease progresses
3. Autonomic distubances
   - Lacrimation

- Salivation (frothing at mouth)
- Perspiration
- Priapism
- Gooseflesh
- Cardiac arrythmias
- Hydrophia and aerophobia

B.Paralytic/dumb rabies

- Seen in partially vaccinated people and people bitten by bats

  Note: - fruit cating bats – major reservoir of rabies virus in west

  Manifestation – flaccid paralysis of bitten limb → f/b quadriparesis → f/b- facial paralysis

iii. Coma and death

- Patient goes into coma and dies in 14 days, after neurological phases
- Patient of dumb/paralytic rabies – may survive for 30 days
- No recovery – death is inerrable

## Diagnosis

1.Direct immune fluorescence (DIF)

- Jo detects nucleoprotein Ag from specimen
- Best specimen – hair follicle at the nape of neck
- Corneal impression smear (may be used) negative in late stage only sensitivity is 30%

- Immunohistochemical stoning – for detection of rabies Ag

2. Virus isolation/culture

a) Specimen inoculated into the brain of sucking mice

↓

Brain is biopsied to look for Negri bodies

b) Cell lines – mouse neuroblastoma cell lines

– baby hamster kidney cell lines

Advantage:

- Growth of virus is faster (2–4 days)
- Growth is detected by antigen detection by direct immune fluorescence

3. Antibody detection – can be done by Elisa, IIF

IN

| Serum | CSF |
|---|---|
| Has little value as: | More value as: |
| • They appear late | • They appear early |
| • Present in vaccinated people | • absent in vaccinated people |

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- antibody detection against viral ribonucleoprotein or glycoprotein can be done by
  - mouse neutralization test (MNT)
  - rapid fluorescence focus inhibition test (RFFT)
  - fluorescent antibody virus neutralization test (FAVN)
  - ELISA

  - IIF
  - CFT
  - Immune peroxidase inhibition assay (IIA)

4.RT – PCR

- Done on saliva, brain tissue
- Most sensitive and specific

5.Post mortem diagnosis – by brain biopsy

- Negri bodies are looked for
  - Intracytoplasmic inclusion bodies with basophilic granules
  - Most site – hippocampus
  - Other- cerebellum, rarely con
- Negri bodies stained by:
  - Histological stains
    - H and E
    - Sella's stain
      (Baric fuchsin with methylene blue and methanol)
  - Better stain than histological stain → immune peroxidase staining method

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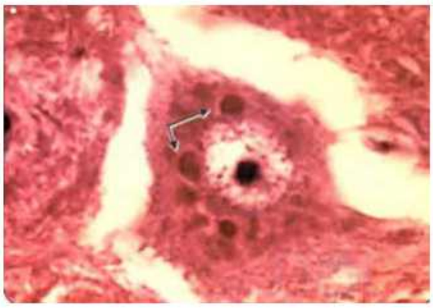

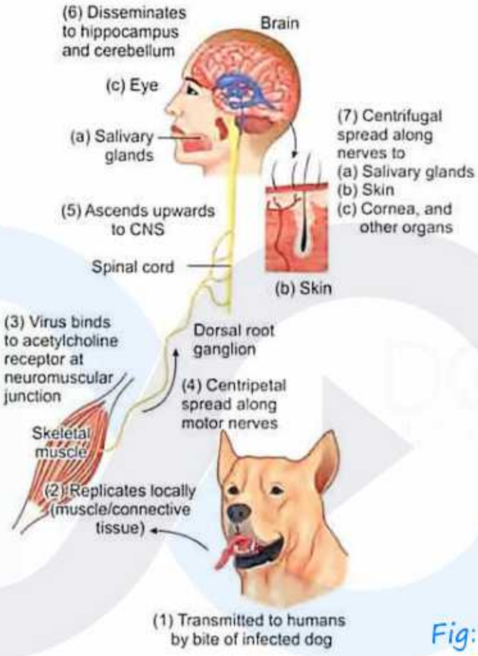
(6) Disseminates to hippocampus and cerebellum
Brain
(c) Eye
(a) Salivary glands
(7) Centrifugal spread along nerves to
(a) Salivary glands
(b) Skin
(c) Cornea, and other organs
(5) Ascends upwards to CNS
Spinal cord
(b) Skin
(3) Virus binds to acetylcholine receptor at neuromuscular junction
Dorsal root ganglion
(4) Centripetal spread along motor nerves
Skeletal muscle
(2) Replicates locally (muscle/connective tissue)
(1) Transmitted to humans by bite of infected dog

Fig: 2

## Treatment

- Isolation
- Maintain hydration, urination
- Sedatives, anti-anxiety drugs.

# POLIO VIRUS

## Picorna viruses 00:00:16

- RNA viruses
- Very small viruses
- Non enveloped
- Icosa- hederal symmetry
- RNA – is positive sense
- 2 groups
  - Enteroviruses

    Mode of infection: feco-oral route
    - Polioviruses
    - Coxsackieviruses
    - Echo viruses
  - Rhino virus

    Inhalation of droplets

## Polio virus 00:03:11

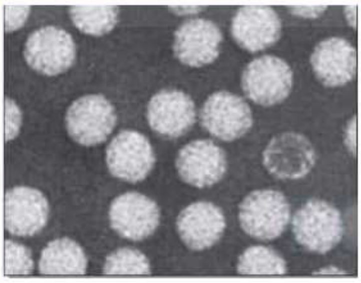

Fig: 1

- These are 3 – antigenic types. Type – 1,2,3 → immunity type specific

| Type – 1 | Type -2 | Type -3 |
|---|---|---|
| Brunhilda and Mahoney strains | Lansing and MEF – 1 Strain | Leant sankett strain |
| Most common cause of epidemic | Most antigenic | No case reported. Hence 2013 |
|  | No case reported. Hence 1999 | Most common type causing VAPP |
|  | Vaccine strains can cause – VDPV |  |

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## Antigens of poliovirus

| Dene antigen | Capsid antigen |
|---|---|
| It is associated with whole vision | It is less specific |
| They are type specific | It is associated with non-infectious verion |
| Anti – D abs are protective | Anti – c abs do not neutralize infectivity |
| Potency of injectable polio vaccine – measured in terms of D – Ag units | |

## Pathogenesis 00:08:18

- I.P: 7-14 days
- Polio virus is transmitted by
  - Feco oral route (most common mode)
  - Respiratory droplet inhalation
  - Rarely conjunctival contact
- After ingestion of virus

↓

Virus multiples locally in intestinal epithelial cells, submucosal lymphoid tissue, tonsils, peyerh patches

↓

Then virus enters the blood – primary viremia

↓

Virus reaches the reticule endothelial system

↓

Then the virus spills into blood – secondary viremia

↓

Now virus reaches brain and spinal cord

- Hematogenous route – most common route by which the virus spreads to brain and spinal cord
- Neural spread – is also seen. The virus can also spread directly through the nerves
  - This occurs during tonsillectomy where virus may spread through the "Glossopharyngeal nerve – present in tonsillar fossa;
- Receptor for polio virus – CD 155

- Final site of action of virus: motor nerve ending anterior horm cells of spinal cord → leads to asymmetric and descending flacid paralysis
- Picorna virus – usually cause "Neuron degeneration" i.e., virus infected neurons undergo degeneration
- Earliest changes – degeneration of "Nissil body"

## Pathological changes:

- They are more extensive than distribution of paralysis

## Features of paralytic poliomyelitis:

- Apart from AFP
  - Meningoencephalitis is present
  - Meningo mycloencephalitis
  - Respiratory failure

## Clinical features:

- 90% infections – asymptomatic / inapparent infection or sub clinical infection
- 5% infections – abortive illness / minor illness/ influenza like symptoms
- 3% infections: aseptic meningitis occurs
- 2% infections: paralytic polia myelitis (AFP + Other features)

## Lab diagnosis 00:17:56

i. Virus – isolation

- Virus can be recovered
  - From throat swabs (up to 1-week of illness)
  - From rectal swabs / faecal samples (up to 6-8 weeks)
- Isolation of virus from CSF/Blood is very rare
- Seawage testing – routinely done to detect wild strain /vaccine strain under routine polio eradication program

  Transport: specimens should be kept frozen during transport to lab
- Cell lines primary monkey kidney

  Growth of virus – detected by
  - Cytopathogenic effect – crenation'/shrinking and degenerationof entire cell line
  - Ag – detection: neutralization with specific and serum, DIF
  - PCR: To detect the VP1 proteins

ii. Ab – detection

- Rise in Ab – titre in paired sera taken at 1-2 weeks interval – suggestive of poliomyelitis
- Both – neutralizing and complement fischg Abs con be detected

iii. Molecular methods

- Real time multiplex RT – PCR:

  - To detect the gene for VP1 protein
  - To differentiate between wild and vaccine strains (VAPP/VDPV)
- Man is the only reservoir
- Period of communicability – patients are infections by shedding the virus in faeces from 7-10 days before the onset of symptoms upto 2-3 days thereafter, sometimes as long as 3-4 months
- No chronic carriers.

# COXSACKIE, ENTERO, ECHO, RHINOVIRUSES

## Coxsackie viruses 00:00:11

- Two groups

1. Group A
    - 1-24 types
    - Except 15, 18, 23
2. Group B
    - 1-6 types

### Coxsackie 'A' virus causes:

- Herpangina/vesicular pharyngitis: characterized by vesicles on fauces, posterior pharyngeal wall
  - Vesicles may breakdown to form ulcers
- Hand – foot mouth disease: characterized by papulovesicular lesions on skin (Hands, feet) + oral cavity
  - Common in kids
- Acute hemorrhagic conjunctivitis: caused by coxsackie A – 24

### Coxsackie – 'B' virus causes:

- Pericarditis
- Myocarditis

- Pleurodynia: Infection of intercostal muscles also known as devil's grip/ epidemic myalgia/ Bornholm's disease
- In pericarditis and myocarditis – patient presents with fever, chest pain, CHF symptoms
  - Dilated cardiomyopathy with global hypokinesia is a dreadful complication
  - This may require a heart transplantation
- Coxsackie – B4 – causes damage to pancreatic cells in mice
  - Suspected to play a role in development of Juvenile diabetes in humans

**Both 'A' and 'B' groups of coxsackie virusescause:**

1. Meningitis (aseptic)
2. Mild paresis
3. Acute flaccid paralysis – similar to polio

- Coxsackie viruses: spread by
  - faeco-oral route
  - respiratory aerosols play a minor role
- Virus multiplies in oropharynx and intestinal tract
- Man: only natural host
- Infections prevalent worldwide (summer and fall)
- Viruses after multiplication – spill into the blood

## Enteroviruses 00:09:53

- serotypes – 70, 71, 72
- serotype – 72 → hepatitis – A – virus
- serotype – 70 causes:
  - acute hemorrhagic conjunctivitis (it is a self-limiting subconjunctival hemorrhage)
- serotype – 71 → causes, hand-foot-mouth disease

### Lab diagnosis:

Specimen: Throat swab, stool, CSF

1. Intracerebral inoculation – into suckling nice
   - in group – A coxsackie viruses: there will be flaccid paralysis
   - in group – B coxsackie viruses: there will be spastic paralysis
   - inoculation of specimen into tissue culture – cytopathogenic effect (CPE) is observed in 5-14 days
2. PCR – VP1 gene detection – sensitive method and specific gene is detected
3. Serology – to detect the neutralizing Abs

## Echoviruses 00:13:49

- E – enteric
- C – cytopathogenic
- H – human
- O – orphan

- Serotypes: 1-33 (except 10, 22, 23, 28) – but all don't cause human diseases
- These viruses are associated with aseptic meningitis, rarely – encephalitis

  Occasionally – rashes, common cold and ocular disease

## Rhinoviruses 00:15:30

- They are acid labile
- Optimal temperature for growth 33°c
- These viruses are confined to URT
- Rhinoviruses – cause common cold (most common infectious disease of humans)
- These viruses have 100 serotypes and the immunity is type – specific
- Hosts:
  - Humans
  - Chimpanzees
  - Gibbons
- Mode of infection: respiratory droplet inhalation
- I.P: 2-4 days

  After this the patient develops:
  - Rhinorrhea
  - Sneezing
  - Nasal obstruction
  - Headache
  - Fever (mild/absent)

- Nasal IgA Ab: responsible for immunity, receptor for Rhinovirus – ICAM – 1

## Diagnosis:

1. Tissue culture: inoculate the virus at 33° c
2. Ag – detection by ELISA
3. PCR

- Cell lines:
  - Primary monkey kidney cell line
  - WI – 38
  - MRC – 5
- Some fastidious strains need organ culture of ferrets or human tracheal epithelium

# ARBOVIRUSES

## Arboviruses 00:00:12

- Arthropod borne viruses
- Vectors are arthopods – Mosquitoes & ticks
- Transmitted by these vectors

## 3 families in arboviruses 00:00:12

**3 families in arboviruses**

**Buniya viruses**

Sandfly fever virus (Vector-sandfly)

Sand fly fever is undifferentiated fever & not dangerous

**Toga viruses**

- Rubella virus
- Chikungunya virus

**Flavi viruses**

- Rubella – is not arthropod borne
- Non arthropod borne toga virus

## Chikungunya viruses 00:02:46

- Vector is aedes aegypti
- It is a togavirus
  - Disease is characterized by fever + arthritis
  - Disease – chikungunya
  - I.P: 3-7days (average: 5days)
- Arthritis in chikungunya
  - Poly articular
  - Migratory
  - Predominantly involves the small joints

### Lab diagnosis

1. Virus isolation
   - It takes 1-2weeks
   - Isolation is done in mosquito cell lines
   - It is done for early diagnosis i.e., 0-7days
2. Serology – serum Ab detection
   - This is the mainstay of diagnosis
   - IgM Ab-appears in 4-days after infection
   - IgG- appears in 2 weeks after infection lasts for years
   - 4 folds or more increase in IgG Ab-helps to make the diagnosis
   - Most commonly used ELISA is MAC ELISA
     - MAC ELISA: for IgM detection
3. RT-PCR:

### i. Yellow fever virus:

- Vector is aedes aegypti
- It is the 1st viral disease of the humans to be discovered
- Yellow fever is prevalent in Central and South America and Africa
- Not preset in India
- I.P: 4-6days
  i. Initial phase: Fever, chills, headache, dizziness, myalgia, backache, nausea and vomiting relative brady cardia
  ii. Severe cases
- Characterized by
  - Hemorrhagic manifestations
  - Platelet dysfunction
  - Hepatitis
- In hepatocytes
  - Torres bodies (intra nuclear inclusion bodies)
  - Renal dysfunction
  - Encephalitis

### Lab diagnosis

- Serology: IgM ELISA
  - It is done after 3days of onset of symptoms
- RT-PCR

### Dengue virus

- 5 serotypes (5th serotype from Bangkok)
- Vector: aedes aegypti
- Disease: Dengue fever/break bone fever/saddle back fever
- Characteristic feature: Fever + myalgia

±

Rash (may or may not be present)

- Complications
  i. Dengue hemorrhagic fever (DHF)
  ii. Dengue shock syndrome (DSS)
- DHF and DSS are because of excessive release of cytokines

### Treatment

- Symptomatic
- Lab diagnosis
  i. First 4-days of fever – NSL Ag by ELISA
  ii. After 4-days – IgM Abs ELISA

## Japanese encephalitis 00:06:38

- In India: Gorakhpur (UP) is the hot belt of JE
- Most cases are subclinical
- Vector: is Culex tritaenorhycle
- Amplifier hosts: Pigs
- Reservoirs: Birds (Herons)

- Horses – are the only animals, to develop symptomatic disease
- Clinical features: Fever + encephalitis
- Severe disease is seen in kids
  - Death (or) permanent neurological sequalae

## Kyasanur forest disease (KFD) virus 00:14:24

- First discovered in India in Shimoga district (Karnataka)
- It causes KFD/monkey fever
- Man-accidental host – causes hemorrhagic fever
- Vector – Hard tick – Haemophysalis spinigera
  - A flavivirus which is not an arbovirus is HCV

## Features of aedes 00:15:50

- One day (day-biters)
- Tiger (tiger mosquito)
- C (Chikungunya)
- R (Rift valley fever)
- Y (Yellow fever)
- D (Dengue)
  - In front of dirty water for cigarettes (cigar shaped eggs)
  - Aedes can transmit dengue virus to its offspring by transovarial transmission

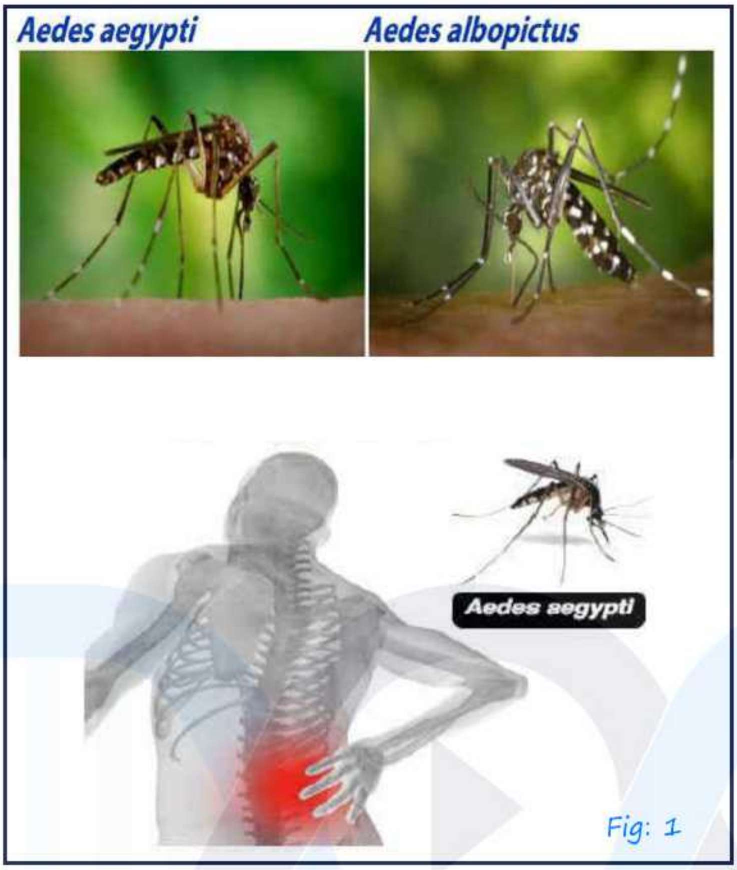
Aedes aegypti
Aedes albopictus
Aedes aegypti

Fig: 1

## Saddle back pattern

- Several days of fever
- Period of improvement
- Followed by several days of resumed symptoms

## Dengue hemorrhagic fever

- Caused by serotype-2
- Bleeding
- Thrombocytopenia
- High mortality

Clinical of dengue

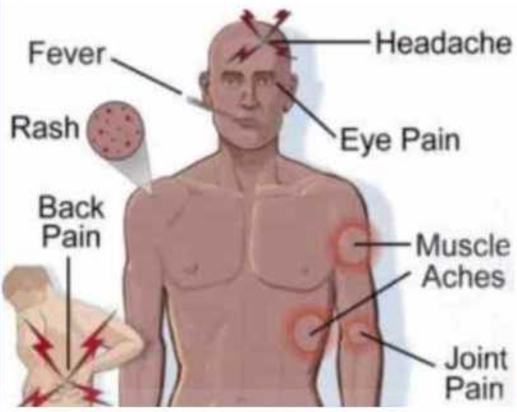
Fever
Headache
Rash
Eye Pain
Back Pain
Muscle Aches
Joint Pain

Fig: 2

WHO case classification of dengue

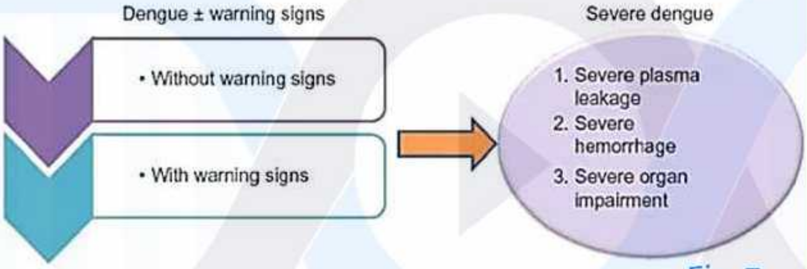
Dengue ± warning signs
Severe dengue
• Without warning signs
• With warning signs
1. Severe plasma leakage
2. Severe hemorrhage
3. Severe organ impairment

Fig: 3

## Probable dengue

- Live-in/travel to dengue endemic area. Fever and two of the following criteria:
  - Nausea, vomiting
  - Rash
  - Aches and pains
  - Tourniquet test positive
  - Leukopenia
  - Any warning signs
- Laboratory confirmed dengue

### Warning sign

- Abdominal pain or tenderness
- Persistent vomiting
- Clinical fluid accumulation
- Mucosal bleed
- Lethargy, restlessness
- Liver enlargement >2cm
- Laboratory: Increase in hematocrit concurrent with rapid decrease in platelet count
  - Requiring strict observation and medical intervention

### i. Severe plasma leakage leading to

- Shock (DSS)
- Fluid accumulation with respiratory distress

### ii. Severe bleeding as evaluated by clinician

### iii. Severe organ involvement

- Liver: AST (or) ALT ≥ 1,000 IU/ML
- CNS: Impaired consciousness
- Heart and other organs

Symptoms of chikungunya

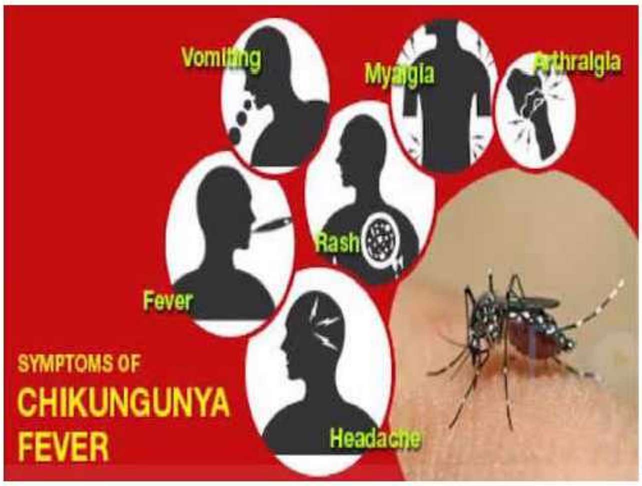
Vomiting
Myalgia
Arthralgia
Rash
Fever
SYMPTOMS OF
CHIKUNGUNYA
FEVER
Headache

Fig: 4

Japanese encephalitis symptoms

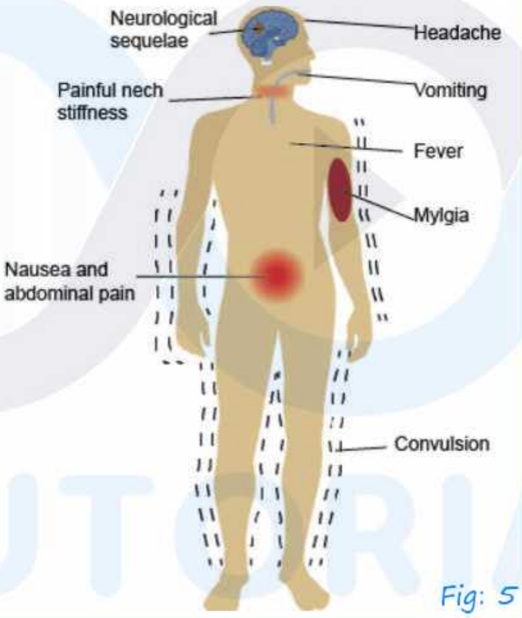
Neurological sequelae
Headache
Painful nech stiffness
Vomiting
Fever
Mylgia
Nausea and abdominal pain
Convulsion

Fig: 5

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# HIV

## Retroviruses 00:00:12

- They have reverse transcriptase (RT) enzyme
- They infect man and animals

  Viral RNA→ RT Viral DNA
- This viral DNA has sticky ends and gets inserted into Host DNA
- This is responsible for latent infections

## Classification 00:02:28

**Retroviridae**

- Oncoviridae
  - HIV – 1
  - HIV - 2
- Lentiviridae
  - HIV – 1
  - HIV – 2

  HTLV → Human t-cell lymphotropic virus
  - HTLV – 1: Causes
    - i. Adult T – cell leukemia
    - ii. Tropical spastic paraparesis
  - HTLV – 2:? Hairy cell leukemia

  HIV → Human Immune Deficiency Virus
  - HIV – 1: prevalent world wide
  - HIV – 2: Prevalent western Africa

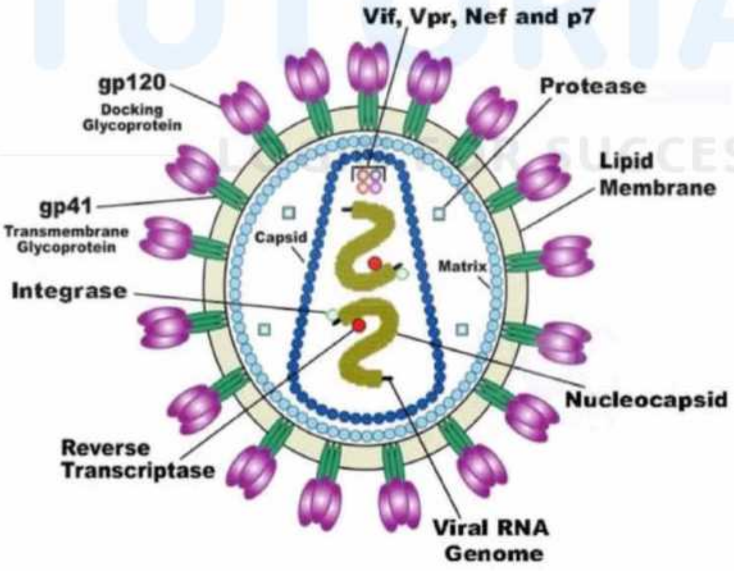
Less prevalent compared to HIV - 1

## HIV - 1 00:05:36

- Divided into groups
  - M
  - N
  - O
  - Most infections are caused by M

### M – Group:

- Again divided into A to K subtypes / clades
- Clade 'C' – most common in India, China and worldwide
- Clade 'A' – common in west Africa
- Clade 'B' – common in Europe, America and Japan


Vif, Vpr, Nef and p7
gp120
Docking Glycoprotein
Protease
Lipid Membrane
gp41
Transmembrane Glycoprotein
Capsid
Matrix
Integrase
Nucleocapsid
Reverse Transcriptase
Viral RNA Genome

- 3 enzymes are present
- The genome of HIV is diploid

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- The virus is spherical
- 100-120nm diameter
- Genome – diploid with 2 molecules of positive sense RNA
- Reverse transcriptase: ← DNA RT RNA
- Integrase: Helps in the integration of viral DNA into Host DNA
- Protease: Cleaves the early/ nascent proteins of virus into mature proteins

### Two proteins:

i. Gp120
  - Binds to receptor for HIV present on Host cells that is CD4
  - CD4 is present on Th cells and macrophages

ii. Gp41
  - Helps in fusion of viral envelope with host cell membrane

- Capsid protein – has core Ag (P24)
- Matrix protein – P17

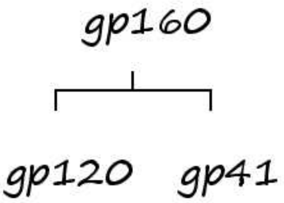
## Genes of HIV 00:16:19

1) Structural genes

   a) gag gene – Encodes for core protein and shell proteins

   b) pol gene: Encodes for RT, Integrase, Protease

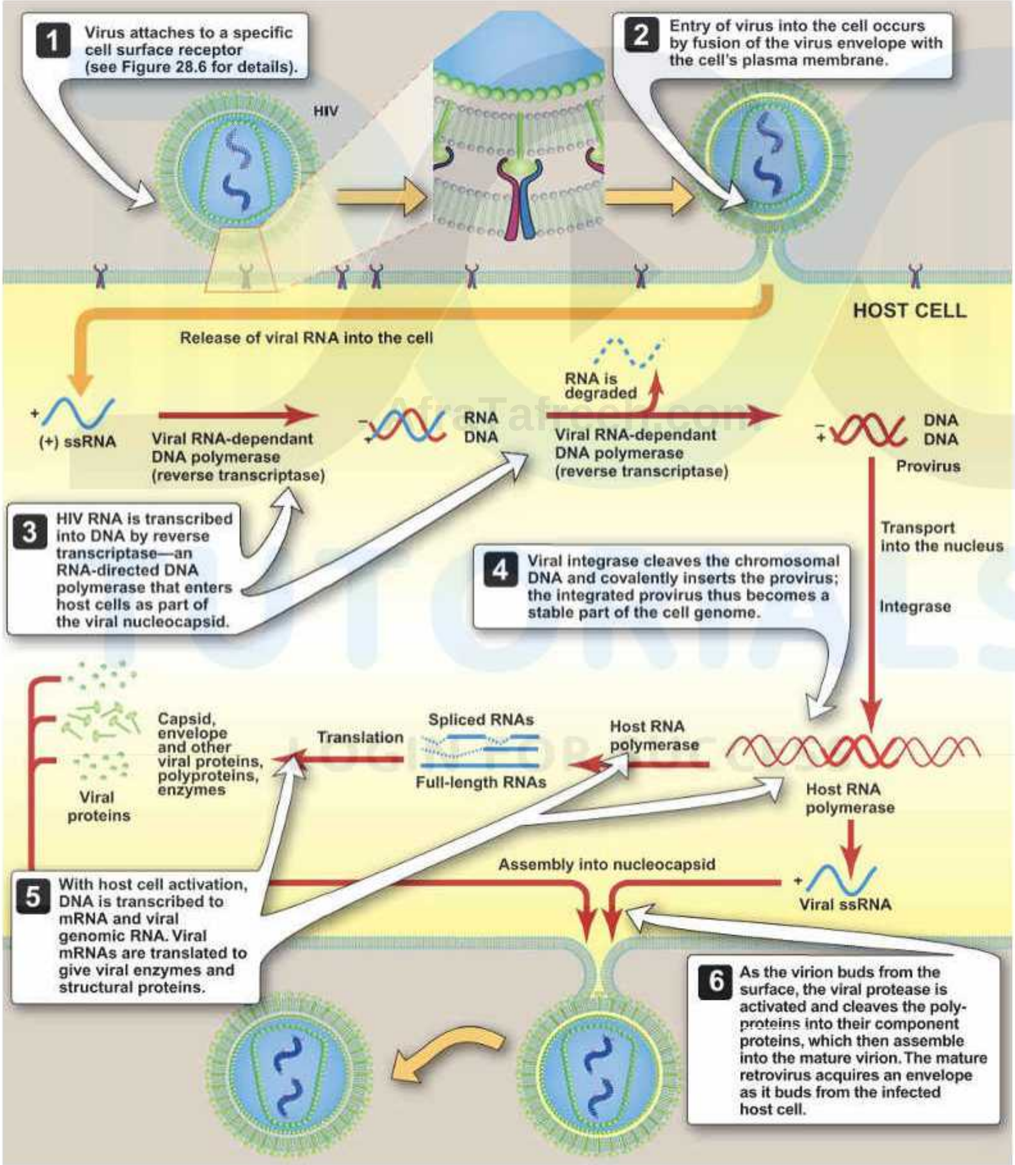
c) env – gene: Encodes for gp-160 protein which is broken down:



2) Nonstructural genes:

- TAT: Transactivates viral transcription
- REV: controls passage of late viral mRNA from nucleus to cytoplasm
- NEF: decreases CD4 and MHC – I expression on virus infected cells
- VIF:
  - Viral infectivity factor
  - Increases infectivity by inhibiting APOBEC3G
  - APOBEC3G Contributes to innate immunity against HIV
  - APOBEC3G – is an apolipoprotein-B which induces mutations in viral DNA
- VPR: It transports viral core from cytoplasm to nucleus of non – diving cells
- Vpu: it increases the release of virions from the cell
- In HIV – 2, VPX is present instead of vpu
- HIV uses a coreceptor:

| CXCR4 | CCR5 |
|---|---|
| Present on $T_H$ cells | Present on macrophages |
| Lysis of $T_H$ cells occurs | Macrophages are APCs, they help in transport of virus |
| $R_4$ strains | $R_5$ strains |



1 Virus attaches to a specific cell surface receptor (see Figure 28.6 for details).

2 Entry of virus into the cell occurs by fusion of the virus envelope with the cell's plasma membrane.

3 HIV RNA is transcribed into DNA by reverse transcriptase—an RNA-directed DNA polymerase that enters host cells as part of the viral nucleocapsid.

4 Viral integrase cleaves the chromosomal DNA and covalently inserts the provirus; the integrated provirus thus becomes a stable part of the cell genome.

5 With host cell activation, DNA is transcribed to mRNA and viral genomic RNA. Viral mRNAs are translated to give viral enzymes and structural proteins.

6 As the virion buds from the surface, the viral protease is activated and cleaves the polyproteins into their component proteins, which then assemble into the mature virion. The mature retrovirus acquires an envelope as it buds from the infected host cell.

HIV
HOST CELL
Release of viral RNA into the cell
(+) ssRNA
Viral RNA-dependant DNA polymerase (reverse transcriptase)
RNA
DNA
RNA is degraded
Viral RNA-dependant DNA polymerase (reverse transcriptase)
DNA
DNA
Provirus
Transport into the nucleus
Integrase
Capsid, envelope and other viral proteins, polyproteins, enzymes
Viral proteins
Translation
Spliced RNAs
Full-length RNAs
Host RNA polymerase
Host RNA polymerase
Assembly into nucleocapsid
Viral ssRNA

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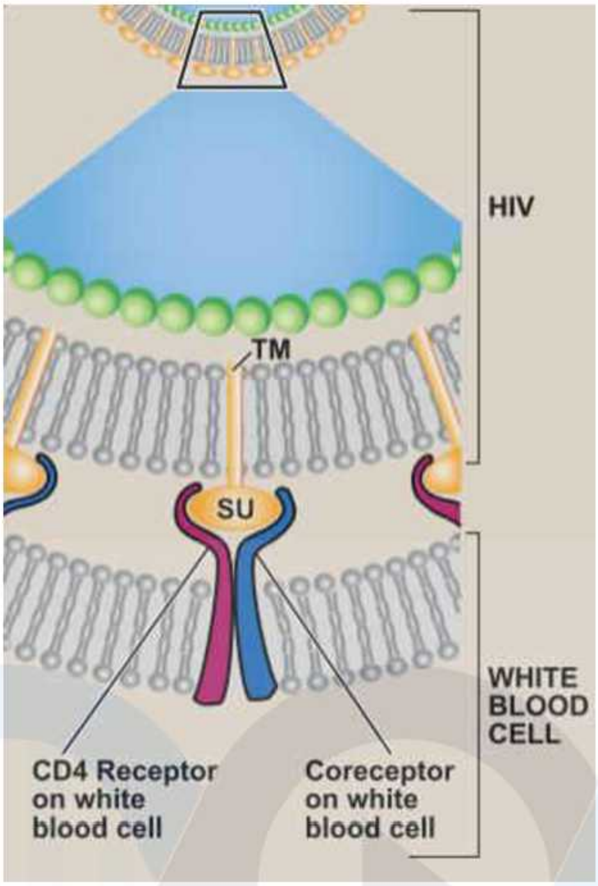
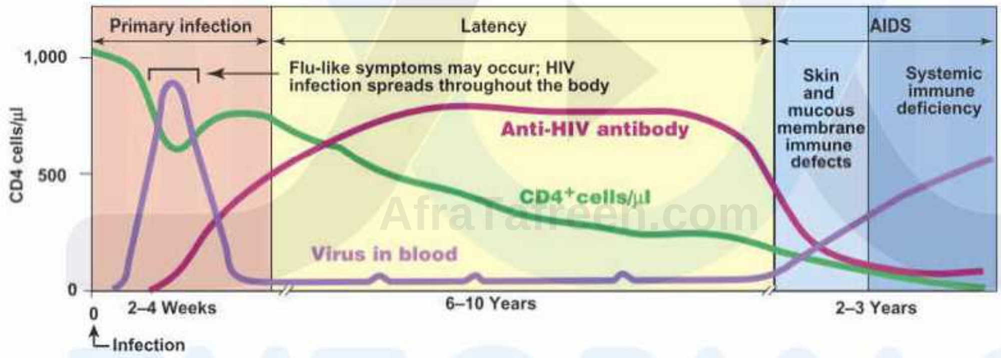

HIV
TM
SU
WHITE BLOOD CELL
CD4 Receptor on white blood cell
Coreceptor on white blood cell

## Modes of transmission 00:28:26

1. Overall, the most common mode is heterosexual intercourse
2. Homosexual intercourse
3. Blood transfusion – most efficient mode (95%)
    - Uncommon mode of transmission
4. Mother to child transmission
    - Before birth (Transplacentally)
    - During birth (During vaginal delivery)
    - After birth (via breast feeding)
    - 50% cases of mother to child transmission occur during birth
5. Needle stick injury (NSI): Risk is 0.3%
    - It is a percutaneous mode

| NSI due to | Risk |
|---|---|
| HIV | 0.3% |
| HCV | 3% |
| HBV | 30% |

- Window period: 2-12 weeks
- It is the time period between infection and seroconversion
- But most cases seroconvert between 3-4 weeks



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## Clinical features 00:36:54

i. Acute phase:

- Mostly asymptomatic
- Few cases develop IMN like syndrome

ii. Period of latency:

- CD4 T cell count decreases
- It is clinical latency but not microbiological latency
- Patient is infected and infectious to others

iii. Acquired immunodeficiency syndrome:

- Occurs when CD4 T – cell count <350/µl

- Patient is prone to opportunistic infections

  Example:

  1. Bacterial
     - Recurrent salmonella septicemia
     - Recurrent bacterial pneumonia
     - Extrapulmonary Tb
     - MAI infection (in the West)
  2. Fungal
     - Candida
     - Cryptococcus neoformans
     - Pneumocystis jirovecii
     - Penicillium marneffi
  3. Parasitic: Diarrhea because of coccidian parasites; Toxoplasma gondii encephalitis; disseminated strongyloidiasis
  4. Viruses: Herpes Viruses

     J.C. virus (Causes progressive multifocal Leukoencephalopathy)

## Diagnosis of HIV infection 00:41:20

1. Screening tests – ELISA and Rapid Tests:
   - Detect Abs
2. Confirmatory test
   - Western blot
3. Gold standard test: is PCR
   - Viral RNA Detection

  - RT – PCR
  - Branched DNA assay
  - NASBA – Nucleic Acid Sequence Based Amplification

4.Real time RT-PCR

- To assess the viral load

## Viral RNA detection

- Most sensitive and specific method of diagnosis
- Positive in window period (PCR is negative for 10-14 days after infection)
- Drug resistant genes can be detected.

## DNA PCR:

- It is preferred for diagnosis of pediatric HIV infection
- Also helpful in making diagnosis in
  - Window period
  - Estimating viral load
  - Also, in detection of genotypes of virus
- DNA PCR also differentiates latent virus infection from active viral transcription

Methods to assess disease progression:

- CD4 T-cell count – is most common
  - Done by flow cytometry
- Viral load estimation by real time RT-PCR – Best method.

# PARASITOLOGY

## Introduction to Parasitology

- Parasites causing infection in humans
- It is divided into
  - Protozoology
  - Heliminthology
- Parasites

  a. Ectoparasites – Resides on the surface of humans – e.g., tics, mite

  b. Endoparasites – Resides inside humans

| Obligate parasites | Facultative parasites | Accidental parasites | Abherrent parasites |
|---|---|---|---|
| • Survive only inside the host<br>e.g.,<br>a) Plasmodium<br>b) Toxoplasma gondii | • free living or inside the host<br>e.g., N. fowleri | • Accidentally infects humans<br>e.g., Echinococcus | • Wanderig parasites<br>e.g.,Toxocara |

## Hosts 00:04:16

| Definitive host | Intermediate host | Paratenic host | Accidental host |
|---|---|---|---|
| • Sexual reproduction | • Asexual reproduction | • Larval stage resides without further development | • Accidentally infecting humans |

## Host – Parasite Relationship: 00:05:55

a. Symbiotic – Both parasite & host benefit from each other

b. Commensal – Parasite is benefitted but the host is not harmed

c. Parasitic – Host is always harmed

# ENTAMOEBA HISTOLYTICA

## E. Histolytica 00:00:12

- They are simple protozoa

  ↓

  Based on ectoplasm & thrusting of ectoplasm

  ↓

  They have pseudopodia (helps in locomotion's)

  - Ectoplasm that us granules
  - It contains
    - RBC's
    - Food vacuoles
    - WBC's
    - Bacteria
  - E. Histolytica is pathogenic to humans
  - E. Hartmanii
  - E. Coli
  - E. Bangladeshi

  ] Not pathogenic to humans

  - Habitat – large intestine

## Morphology 00:03:00

a) Trophozoid

b) Pre – cyst

c) Cyst

- Immature
- Mature

  ↓

  Quadri nucleate cyst (Infective form)

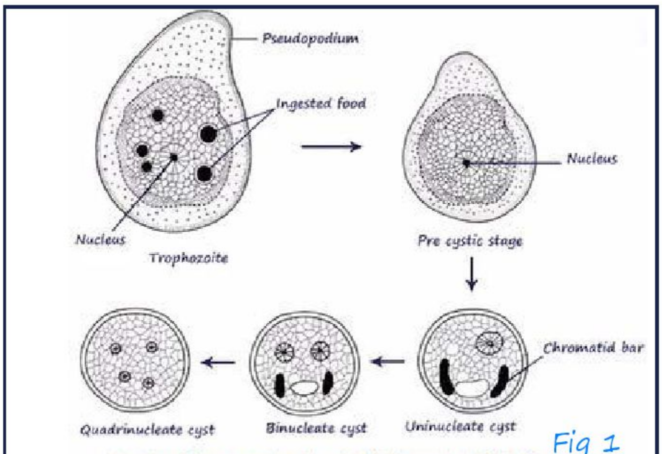

Pseudopodium
Ingested food
Nucleus
Trophozoite
Nucleus
Pre cystic stage
Chromatid bar
Uninucleate cyst
Binucleate cyst
Quadrinucleate cyst

Fig 1

Trophozoite, precyst and cyst of Entamoeba histolytica

- Outer ectoplasm is clear
- Inner endoplasm contains RBC's, ingested food, vacuoles
- In trophozoite – it has a cartwheel appearance
- Presence & non – pathogenic species RBC is present in pathogenic species

- Presence of karyosome is central in Entamoeba histolytica
- Trophozoites
  - Only seen in acute dysentery
  - Indicator of active infection
  - It divides by binary fission
  - It cannot survive for a long time
- Pre – cyst
  - It has a cart wheel appearance
  - Food vacuole is absent
  - Thick cyst wall is present

**Infective stage + diagnostic stage – Quadri nucleate cyst**

## Life cycle 00:09:30

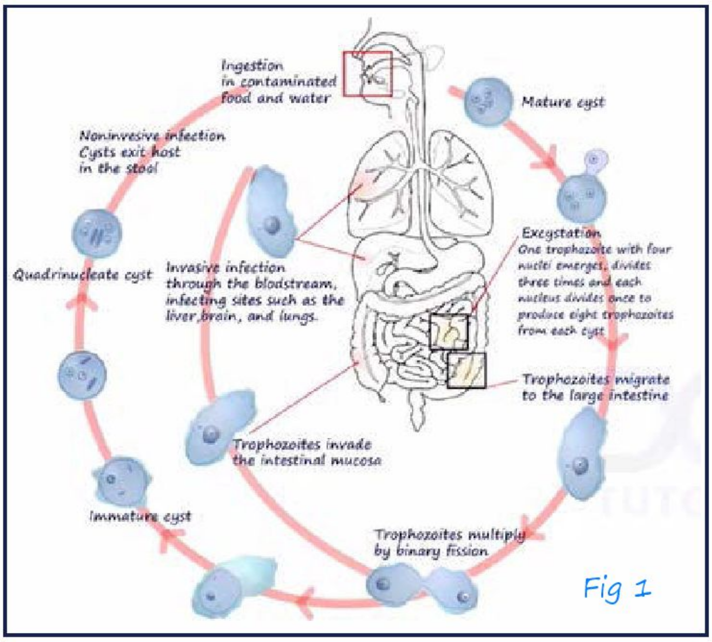

Ingestion
in contaminated
food and water
Mature cyst
Noninvesive infection
Cysts exit host
in the stool
Quadrinucleate cyst
Invasive infection
through the blodstream,
infecting sites such as the
liver,brain, and lungs.
Excystation
One trophozoite with four
nuclei emerges, divides
three times and each
nucleus divides once to
produce eight trophozoites
from each cyst
Trophozoites migrate
to the large intestine
Trophozoites invade
the intestinal mucosa
Immature cyst
Trophozoites multiply
by binary fission

Fig 1

- Route – feco -oral
- Excystation takes place in small intestine
- Small intestine – Meta trophozoites
- The cysts are constantly shed in stools

## Lab diagnosis 00:13:15

1) Intestinal Amoebiasis

a)Stool examination

- Leukocytosis
- Charcot – Leyden crystals
- Quadri nucleate cyst in stool
- Trophozoites in adult dysentery

- Patient gives h/o stool with blood & mucus
- It is foul smelling & does not adhere to container
- No h/o fever

## b) Culture 00:16:17

| **Axenic culture** | **Culture with presence of** |
|---|---|
| • E.g., diamonds media (Absence of bacteria)<br>• For drug susceptibility<br>• For isolation of antigen | • Bacteria<br>• Starch & other nutrients<br>Polyxenic culture<br>• E.g., Nelson's NIH media |

## c) Serological diagnosis 00:18:07

| **Lectin antigen** | **Virulence factor** |
|---|---|
| • Used both for detection and coproantigen detection in stool<br>• Same antigen is detected in ELISA | • Lectin antigen – most important<br>• Cystine protease<br>↓<br>Responsible for ulcers in amoebiasis |

- ELISA for antibodies
  - Antibodies are elevated & hence cannot differentiate between active and past infection
- In PCR – multiplex PCR

  ↓

  - Bio fire (test for multiple organisms) method

## Intestinal Amoebiasis

- With the help of histopathology – flask shaped ulcers are seen stomach
  ↓
  Caused by the effect of protease in the stomach
- Ulcers are most commonly seen in ileo – caecal region
- Flask shaped ulcers has a narrow neck & broad base
- Most common site – Rectosigmoid region
- Amebomas – Granulomatous tissue
  - Mimics carcinoma (rectosigmoid region)

---

## Extra – intestinal 00:23:37

1) Liver
   - Affects liver the most due to transport of the organism through portal system
   - In liver, it produces lesions that are singular/multiple usually seen in right lobe of liver
   - PUS →Anchovy sauce appearance
   - USG guided aspiration of pus
   - Microscopy – Trophozoites can be seen
2) Lungs – pleuritis
3) Heart – pericarditis
4) Local infection
5) Blood stream → kidney

## Treatment 00:26:23

- Metronidazole
- Tinidazole
- Ornidazole

+

Paromomycin

## Difference between E. Histolytica & E. coli 00:27:17

| | **E. Histolytica** | **E. Coli** |
|---|---|---|
| • Trophozoite | • RBC's present | • RBC's absent |
| | • Karyosome is central | • Karyosome is eccentric |
| Cysts | • Quadri nucleates | • 8 nuclei |
| Lectin antigen | • ⊕ | • Absent |
| | • Isoenzymes are specific | • Iso – enzyme is non – specific |
| PCR | • Specific | • Non – specific |

# FREE LIVING AMOEBA

## Free living amoeba

- Often found in soil
- Usually not pathogenic but tend to become pathogenic under certain conditions
  - B – Balamuthia Mandrillaris ⎤ GAE
  - A – Acanthamoeba. Culbertsoni ⎦
  - N – Negleria fowleri→ PAM
  - GAE – Granulomatous amoebic encephalitis
  - PAM – primary amoebic & meningoencephalitis

---

## Negleria fowleri 00:02:15

---

- Morphological forms
  - Cysts
  - Trophozoites
    - Amoebic
    - Biflagellate
- Cysts are produced in unfavorable condition
- Trophozoites
  - Amoebic trophozoite (infective form)
  - Biflagellate trophozoite
    - Move to newer water bodies
- In CSF – No cysts or biflagellate trophozoite
- Negleria fowleri is thermophilic

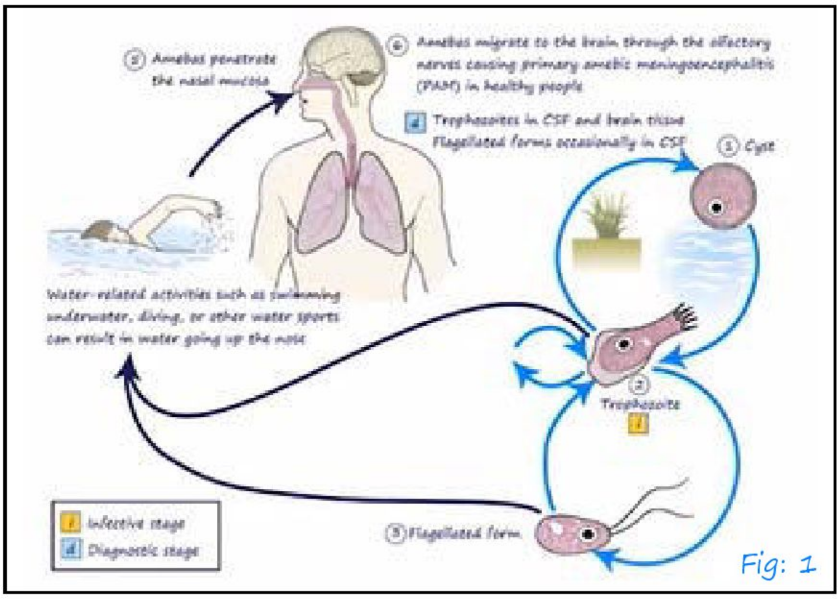

⑤ Amebas penetrate the nasal mucosa
⑥ Amebas migrate to the brain through the olfactory nerves causing primary amebic meningoencephalitis (PAM) in healthy people
d Trophozoites in CSF and brain tissue
Flagellated forms occasionally in CSF
① Cyst
Water-related activities such as swimming underwater, diving, or other water sports can result in water going up the nose
② Trophozoite
i
③ Flagellated form
i Infective stage
d Diagnostic stage

Fig: 1

- Very common in freshwater ponds, lakes
- Incubation period – 2-7days

## Lab diagnosis 00:06:35

- CSF findings – similar to pyogenic meningitis

  a)↑ proteins

  b)↓ glucose

  c) Neutrophilic in CSF
- CSF – Trophozoites (amoeboid) are seen in CSF

## Treatment 00:08:55

- Drug of choice – Amphotericin – B

# Acanthamoeba 00:10:08

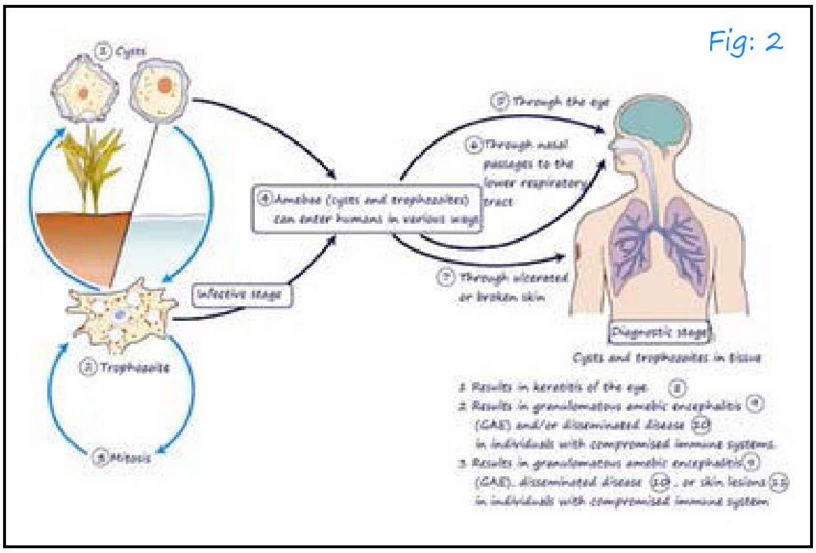


Fig: 2
① Cysts
② Trophozoite
③ Mitosis
Infective stage
④ Amebae (cysts and trophozoites) can enter humans in various ways
⑤ Through the eye
⑥ Through nasal passages to the lower respiratory tract
⑦ Through ulcerated or broken skin
Diagnostic stage
Cysts and trophozoites in tissue
1 Results in keratitis of the eye ⑧
2 Results in granulomatous amebic encephalitis ⑨ (GAE) and/or disseminated disease ⑩ in individuals with compromised immune systems.
3 Results in granulomatous amebic encephalitis ⑨ (GAE), disseminated disease ⑩, or skin lesions ⑪ in individuals with compromised immune system

a) Cyst

b) Trophozoite – infective stage

↓

Acanthopodia – spinous shaped pseudopodia

- Mode of spread
  - a) Eyes
  - b) Respiratory passage
  - c) Ulcerated skin
- Eyes
  - Causes acanthamoeba keratitis
  - Causes GAE from spread
- CSF findings
  - Normal or slight decrease in glucose
  - Lymphocytes in CSF
  - Both cysts & trophozoites in CSF & brain tissue

- Amoebic keratitis
  - Diagnosis

    a)Corneal scrapings

    ↓

    Trophozoites are seen

    b)Axenic culture &

    c)Nutrient agar with E. coli

    d) Calcofluor white → examination

## Balamuthia mandrill Aris 00:15:25

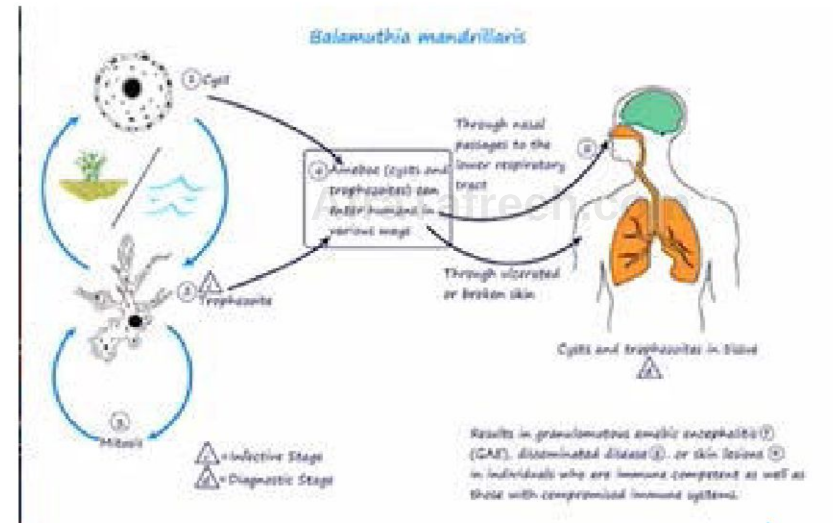

Balamuthia mandrillaris
① Cyst
② Trophozoite
③ Mitosis
④ Amebae (cysts and trophozoites) can enter humans in various ways
Through nasal passages to the lower respiratory tract
Through ulcerated or broken skin
⑤
Cysts and trophozoites in tissue
= Infective Stage
= Diagnostic Stage
Results in granulomatous amebic encephalitis ⑦ (GAE), disseminated disease ⑧, or skin lesions ⑨ in individuals who are immune competent as well as those with compromised immune systems.

Fig: 3

- Causes GAE
- Mode of spread – Nasal passage & ulcerative skin
- Cysts – have refractile cell wall
- Trophozoites – infective stage
- It affects in immunocompetent & immunocompromised
- Diagnosis
  - Cysts & Trophozoites in brain tissue

# GIARDIA

## Giardia lamblia

- It is a protozoan that resides in small intestine
- Other protozoan in SI

  a)Isospora

  b)Cryptosporidium

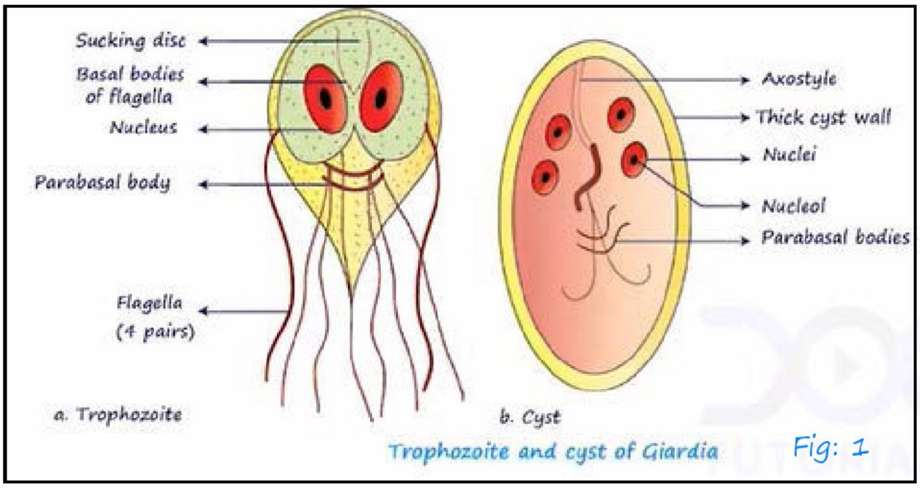
  c)Cyclospora

- Protozoa with whip like flagella

| Luminal flagellates | Hemoflagellates |
|---|---|
| a)Giardia | a)Leishmania |
| b)Trichomonas | b)Trypanosoma |

1) Infective dose 10 – 100 cysts

2) Motility – Trophozoite – falling leaf motility


Sucking disc
Basal bodies of flagella
Nucleus
Parabasal body
Flagella (4 pairs)
a. Trophozoite
Axostyle
Thick cyst wall
Nuclei
Nucleol
Parabasal bodies
b. Cyst

Trophozoite and cyst of Giardia Fig: 1

a)Trophozoite – Tennis racket shaped or pyriform

- Adherence is due to sucking disc
- 1 pair of nucleus

b)Cyst – ellipsoid cyst

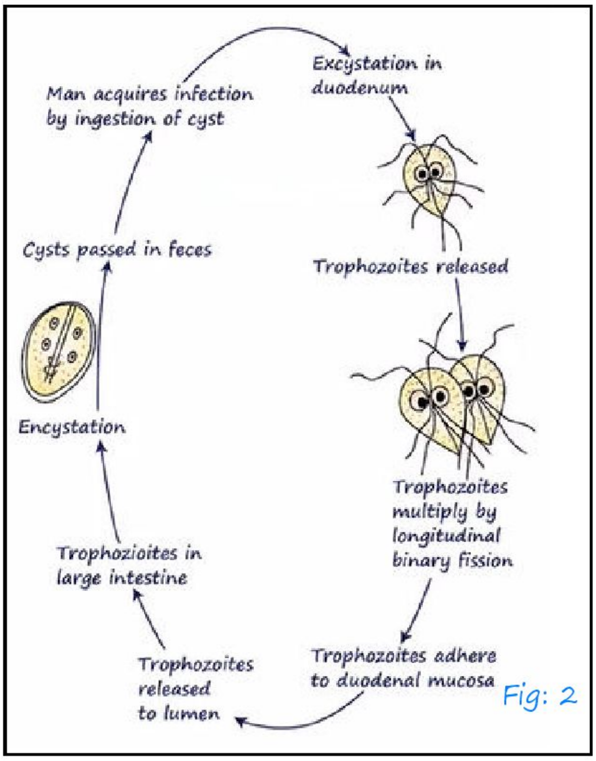

Man acquires infection by ingestion of cyst
Excystation in duodenum
Trophozoites released
Trophozoites multiply by longitudinal binary fission
Trophozoites adhere to duodenal mucosa
Trophozoites released to lumen
Trophozioites in large intestine
Encystation
Cysts passed in feces

Fig: 2

- Spread – Feco-oral route

AfrozTafreeh.com

## Pathogenesis 00:05:40

- Here, it does not invade the mucosa
- It is because of vilus alteration
- Presence of malabsorption of vitamins, fat (steatorrhea)
- Virulence factors
  - Surface antigen – VSSP's
    ↓
    Variant specific surface protein
- Differential diagnosis
  - Patient present with stool with mucus but no blood
  - Affects children most commonly

## Lab diagnosis 00:08:24

1) Stool examination
   - Cysts in stool (ellipsoid cyst)
2) Enterotest
3) Antigen & antibody detection →ELISA
4) PCR – multiplex PCR

## Treatment 00:10:25

- Metronidazole
- Tinidazole
- Ornidazole
- Paromomycin

# TRICHOMONAS VAGINALIS

## Trichomonas species

a)Trichomonas vaginalis -STD

b)T. Tenex ] Commensals

c)T. Hominis

## Trichomonas vaginalis 00:01:11

- It only has a trophozoite stage

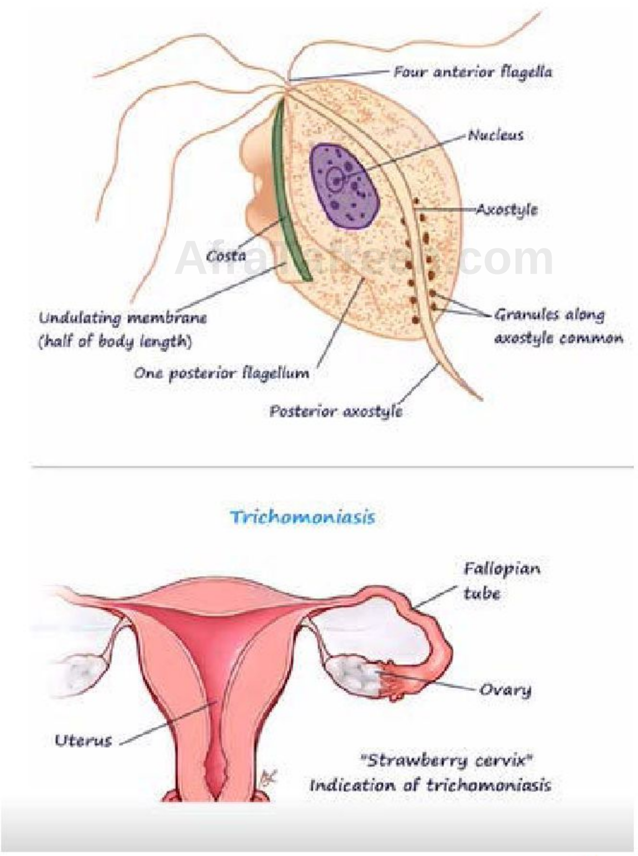

Four anterior flagella
Nucleus
Axostyle
Costa
Undulating membrane (half of body length)
Granules along axostyle common
One posterior flagellum
Posterior axostyle
Trichomoniasis
Fallopian tube
Ovary
Uterus
"Strawberry cervix" Indication of trichomoniasis

Fig: 1

- Siderophillic granules are commonly present along axostyle
- Trophozoite is infective & diagnostic stage as no cyst is present

## Life cycles 00:03:39

- It is sexually transmitted
- Women-vagina
  - Cervix
  - Urinary bladder
- Men
  - Anterior urethra
  - Prostate

## Clinical features 00:04:25

- Greenish discharge
- Frothy
- Associated with pruritis
- Urethral discharge

## Complication 00:05:25

a)Cervicitis

b)Endometritis

c)Salpingitis

- Strawberry cervix is very specific of trichomonas vaginalis

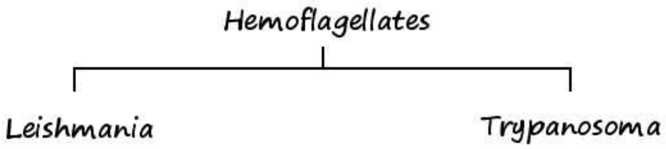
## Lab diagnosis 00:06:15

1. Wet mount – Trophozoites showing ' Twitching motility'
2. Culture – Gold standard
   - Plastic envelope medium(PEM)
3. ELISA – Antigen & antibody detection
4. PCR – multiplex PCR

## Treatment 00:08:20

- Contact tracing – Both partners are treated simultaneously
- DOC – metronidazole
- Preventive
  - Barrier methods of contraception
  - Contact trace & treatment

# LEISHMANIA



## Leishmania 00:00:35

- Has animal reservoir
- Disease: Leishmaniasis
- Zoonotic disease

### 1.L. donovani

- Causes visceral lesimaniasis/ kala azar/ Indian kala azar
- Strict human pathogen, not a zoonotic disease
- Vector: Sand fly/ Phlebotomus argentipes

### 2.L. tropica

- Causes cutaneous leishmaniasis/ Delhi boil/ Baghdad boil/ Oriental sore
- Vector: Sand fly/ Phlebotomus sergentii

### 3.L. brasiliensis

- Causes Mucocutaneous leishmaniasis/ espundia
- Vector: Sand fly

### 4.L. mexicana:

- Causes chiclero's ulcer (type of culaneous leishmaniasis)
- Vector: Sand fly

- Animal reservoirs:
  - Foxes
  - Rodents
  - Dogs

- Leishmaniasis
  - Old world (Asia) → Phlebotomus is prevalent
  - New world – Central & South America → Lutzomyia

## L. donovani 00:06:23

- 2 forms:

| Amastigote form | Promastigote form |
|---|---|
| Aflagellated | Flagellated |
| Present in man-in Reticulo endothelial system (RES)<br>- Bone marrow, liver, spleen | Present in sand fly, mainly in mid-gut |

- Amastigote form:
  - Present in RES
  - RES – rich in macrophages

## LD body

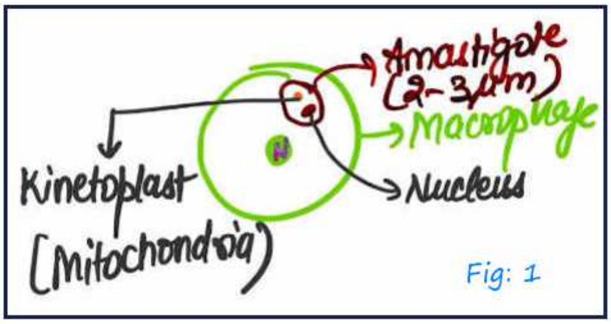

Amastigote (2-3µm)
Macrophage
Nucleus
N
Kinetoplast (Mitochondria)

Fig: 1

- Kinetoplast is the mitochondria of the organism
- Macrophage filled with amastigoles is called as **LD body**

## Life cycle

Sand fly bites the infected human
↓
Picks up amastigotes, LD bodies
↓
Amastigotes change to Promastigotes
↓
Fly becomes infected; when bites another human, it injects the promastigotes into skin
↓
Enter blood
↓
Promastigotes invade monocytes, macrophages where they change to amastigote forms
↓
Multiply & released out

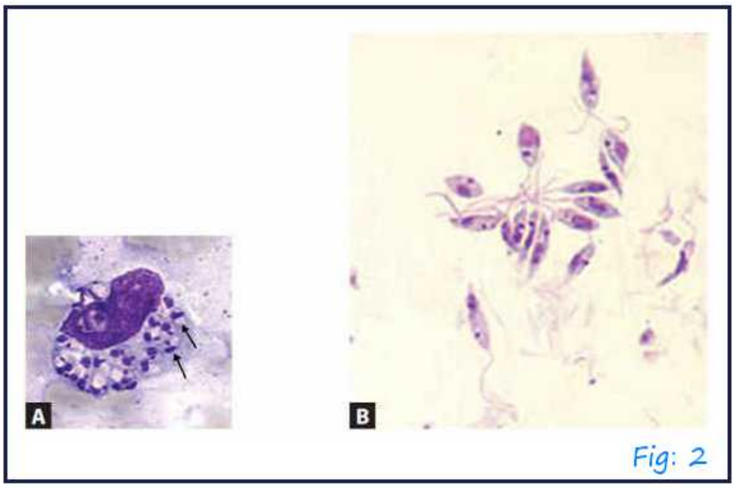
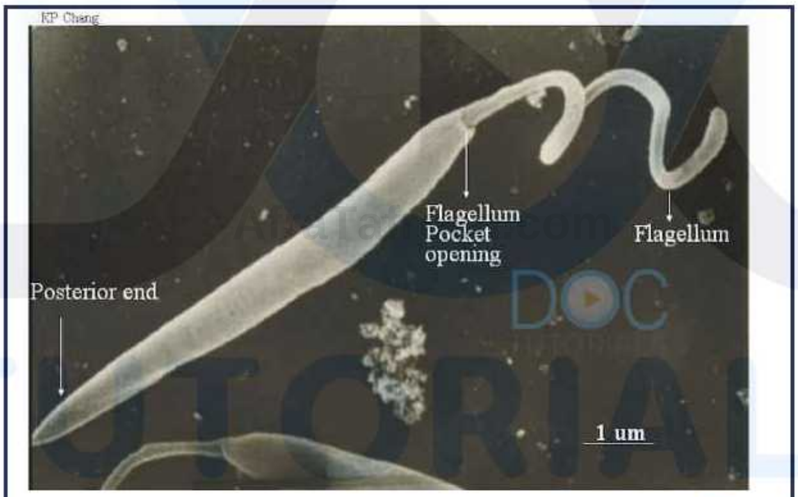


Fig: 2

a. Amastigote

b. Promastigote


KP Cheng
Flagellum Pocket opening
Flagellum
Posterior end
1 um

Electron microscopy → promastigotes

Fig: 3

## Clinical features

- I.P: Long, variable (1-6months)
- Patient presents with
  - Fever
  - Hepatosplenomegaly
  - Complications
    - i. Intestinal haemorrhage
    - ii. Secondary bacterial peritonitis

## Diagnosis 00:16:32

### Non-specific tests

- Anaemia
- Thrombocytopenia
- Leukopenia
- Hyper-gammaglobulinemia can be detected by
  - Napier's aldehyde test
  - Chopra's antimony test
- Reversal of A:G ratio (Normal: 1.7:1)

### Specific tests

- Rests on parasitological diagnosis
- Specimen
  - Splenic aspirate (more sensitive)
  - Liver biopsy
  - Bone marrow aspirate (most common specimen)

a. Staining of specimen: Giemsa stain – see LD bodies

b. Culture on:

1. NNN (Novy McNeal Nicolle Medium)
   - Biphasic medium with 2 parts salt-agar
     +
     1 part defibrinated rabbit's blood
2. Schneider's medium
3. Toby's medium

- All these media, provide an environment similar to sandfly
- Promastigotes are seen on microscopy

c. Ab-detection by

  - ELISA, DAT (Direct agglutination test)
  - Indirect fluorescent antibody test

d. Rapid test: Immunochromatographic Test (ICT): Detects antibodies against rk39 Ag (Recombinant Kinetoplast)

## Treatment

- **DOC:** Pentavalent antimony compounds
- **Miltefosine:** Only oral drug for kala azar
- In resistant cases: **Amphotericin B**

# TRYPANOSOMA

## Trypanosoma 00:00:10

- It is a hemoflagellate
- Not found in INDIA
- It is classified into 2-species T. brucei & T. cruzi

| | T. brucei | T. cruzi |
|---|---|---|
| **Disease** | Sleeping sickness/ African trypanosomiasis<br>Prevalent in Africa | Chaga's disease/ American trypanosomiasis<br>Prevalent in South America & Central America |
| **Vector** | Tse-Tse fly/Glossina species | Reduviid bug/kissing Bug/Assasin bug |
| **Lesion** | Ulcer at the site of bite<br>This ulcer is known as Trypanosomal chancre<br>↓<br>Then parasite enters Blood circulation<br>↓<br>Reaches CNS<br>↓<br>Causes encephalopathy (Sleeping sickness)<br>2 important signs<br>i. Winter bottom's sign – posterior cervical lymphadenopathy<br>ii. Krenalde's sign: Delayed perception of pain | Swelling at the site<br>This swelling is known as CHAGOMA/ Romana's sign<br>Ramana's sign: Periorbital edema<br>From swelling, Parasite enters blood<br>Causes<br>Megacolon<br>Megaesophagus<br>Cardiomegaly |

- T. brucei: shows antigenic variation

## Diagnosis:

1. Peripheral blood smear:
   - To see trypomastigotes (flagellated forms) (Figure1)
   - Diagnosis of Chaga's disease – "Xenodiagnosis"

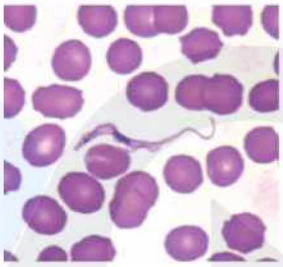


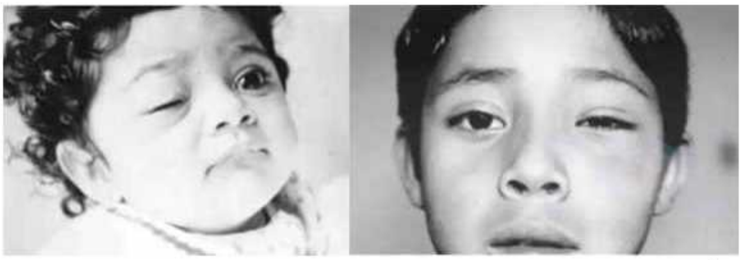
Fig: 1

2. Antibody in serum, CSF- By ELISA, IIF, Card agglutination test
3. Antigen in serum, CSF by ELISA
4. PCR
5. Culture in KIVI medium

## Treatment

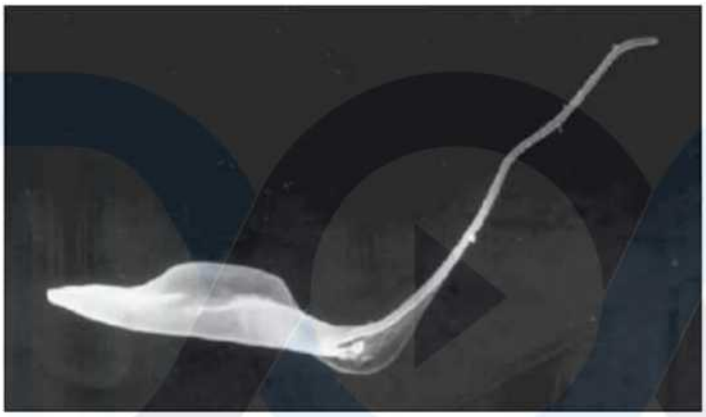
- Early stage: Pentamidine, suramin
- Late stage: Eflornithine, melarsoprol

# Chaga's disease



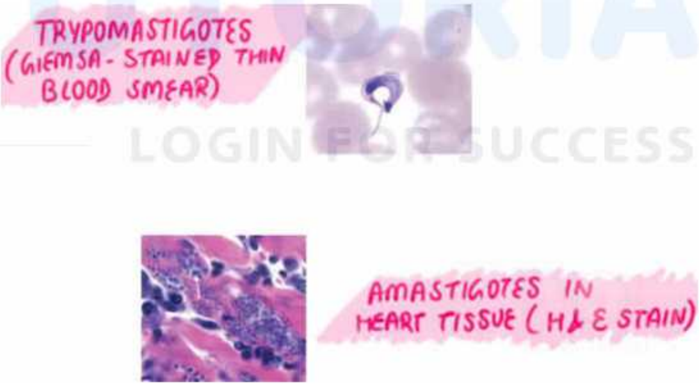
Romana's sign

Fig: 2



Scanning electron micrograph of a trypomastigote of T.cruzi

Fig: 3


TRYPOMASTIGOTES (GIEMSA-STAINED THIN BLOOD SMEAR)
AMASTIGOTES IN HEART TISSUE (H & E STAIN)

Treatment of chaga's disease – Nifurtimox & benznidazole

Fig: 4

# PLASMODIUM

## Plasmodium 00:00:10

- Non motile protozoan
- Causes malaria

**Species:**

- P.vivax – most common cause of malaria in India.
- P.falciparum – fatal disease (most severe malaria)
- P. malariae
- P. ovale – not found in India
- P. knowlesi – in monkeys

**P. vivax**

- Malaria is referred as **Beningn Tertian Malaria.**
- Less severe, fever spike occurs on Day – 3 (after 48 – hours)

- P.falciparum – **Malignant Tertian Malaria**
  - Severe form
- P.malariae – causes **Quartan malaria**
- P.ovale: - causes **Ovale Tertian Malaria**

## Life Cycle 00:04:07

Definitive host Is female anopheles mosquito

- Definitive host means one in which sexual cycle takes place
- The sexual cycle in female anopheles mosquito is called "SPOROGONY"
- Sporozoites are formed during sporogony
- Sporozoites – present in the mouth part of mosquito
  - They are infective forms for humans.
- Only female mosquito, not male – because female mosquito needs blood meal for egg maturation.

**Intermediate host: Asexual cycle of parasite takes place.**

- Is man
- Asexual cycle in man known as Schizogony.
- Schizonts are formed during schizogony.
- Schizonts – are multi-nucleated structures.

**Mosquito**

When it bites humans, spindle shaped sporozoites are injected

into bloodstream

↓

Reach liver

↓

Pre/Exoerythrocytic Schizogony

(before invading RBCs)

↓

In hepatocyte: Sporozoite → Early trophozoite/Ring stage (ET)
↓
Late Trophozoite (LT)
↓
Released out by rupture of hepatocyte ← Merozoite ← Hepatic Schizont

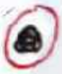
↓

Merozoites – Circulate in the blood stream

- Some merozoites – invate RBCs → Initiate **Erythrocytic Schizogony**

## In RBC

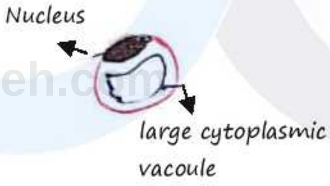
Merozoites



↓

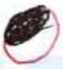
Early trophozoite/ring form/ring stage

(diamond on a ring)



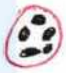
↓

Late Trophozoite



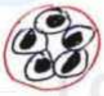
↓

Erythrocytic schizont



↓

merozoites



↓

Released out by rupture of RBC

Some invade the other RBCs & restart the erythrocytic cycle.

Onset of clinical features in the patient.

Some merozoites transform into:

Male gametocyte
(Microgametocyte)

Female gametocyte
(Macrogametocyte)

DOC TUTORIALS
LOGIN FOR SUCCESS

- Male & Female gametocytes are Infective forms for mosquito.
- If these are not picked up by mosquito, then they dsintegrate.
- Sporogony occurs in mosquito.
- Microgametocyte → Macrogemotocyte

| Microgametocyte | Macrogemotocyte |
|---|---|
| ↓ changes to | ↓ Changes to |
| Microgamete | Macrogamete |

- Both Microgamete and macrogamete fuse to form:

ZYGOTE

↓ Transforms to

Ookinete (Transient motile form)

↓

Oocyst

↓

Multiplies to give rise to multiple Spindle shaped,

SPOROZOITES

↓

Released into the body cavity of the mosquito,

some reach the mouth parts

- Life cycle of plasmodium – called as CYCLOPROPAGATIVE LIFE CYCLE

## Complications of Falciparum malaria: 00:23:44

i. Pernicious malaria: parasitemia > 5%

ii. Cerebral malaria: Encephalopathy

iii. Black water fever – Intravascular hemolysis

- Black water fever, usually occurs on 2nd attack by P.falciparum, it is a hypersensitivity reaction.
- Does not occur after 1st infection.

iv. Metabolic acidosis

v. Algid malaria: Malaria + shock

vi. Hypoglycemia

- **Splenic rupture:** very rare complication of malaria.
  - → most common cause is P. vivax
- **Nephrotic syndrome:** by P. malariae.

**Reactivation Malaria**

| Relapse | Recrudescence |
|---|---|
| • seen in<br>P. Vivax<br>P. Ovale | • seen in<br>P. Falciparum<br>P. Malariae |
| • Relapse – occurs because of Hypnozoites in liver | • Occurs because of Drug resistant forms present in blood |

## Diagnosis of Malaria 00:30:16

**I. Peripheral Blood Smear: Gold standard method.**

| Thick smear | Thin smear |
|---|---|
| ↓ | ↓ |
| Is for diagnosis | Is for speciation. |

| | P.v | P.f | P.m | P.o |
|---|---|---|---|---|
| % of RBC's Involved | <2% | >2% | <2% | <2% |
| Stage of RBC involved | Young RBCs | All stages | Old RBCs | Young RBCs |
| Stippling | Schuffner's dots | Maurer's dots | Zeimann's dots | James dots |
| Early trophozoite/Ring forms | ✓ | ✓ | ✓ | ✓ |
| Late Trophozoite | ✓ | X | ✓ | ✓ |
| Schizont | ✓ | X | ✓ | ✓ |
| Gametocyte | ✓ | ✓ | ✓ | ✓ |

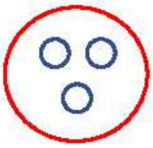
Merozoites – too tiny to be seen on peripheral blood smear

- In P.falciparum: late trophozoite, schizonts – are attached to the endothelium of capillaries of internal organs – wherever they are formed.
- This is known as CYTOADHERENCE
- Cyto-adherence leads to sequestration of the parasite

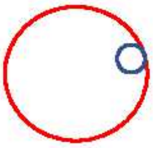
## Stippling

- pink/red dots on surface of infected RBCs. They represent membrane-bound structures in infected RBCs which help in protein transport from parasite to erythrocyte surface.

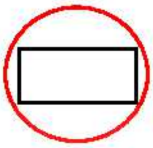
RBC



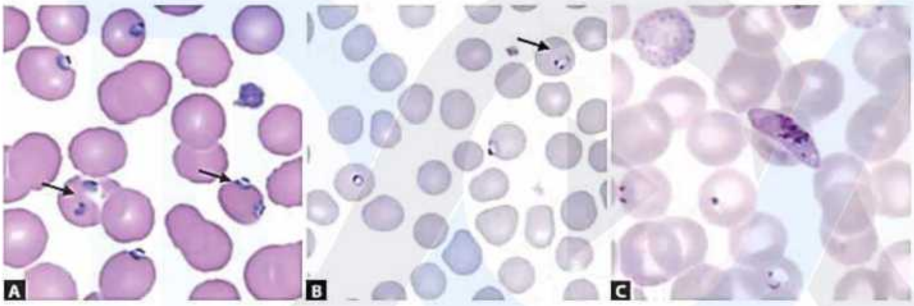
→ If multiple rings are present → p. falciparum



→ It is appleque form/accole form



→ Band form: It represents late trophozoite form of P. malariae

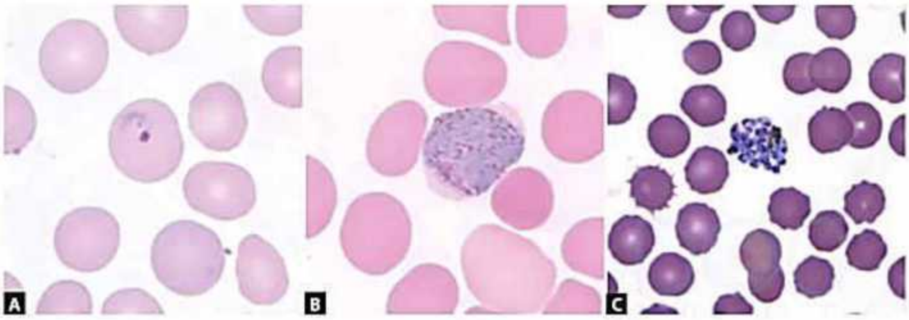


P.falciparum – A, B, C

Fig: 1

Thin blood smear showing different forms of plasmodium falciparum

(A) Multiple ring form and accole form; (B) Double dot (Head phone shaped ring form; (C) Gametocyte

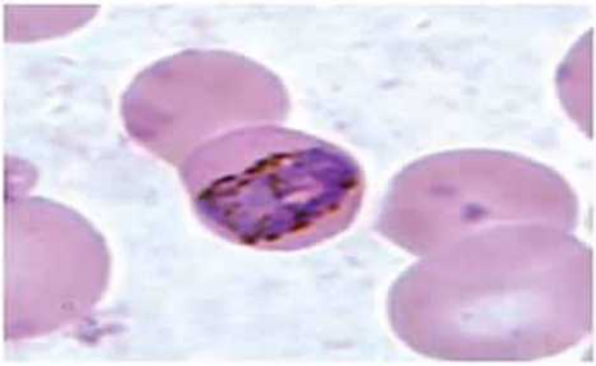


P.vivax

Fig: 2

Thin blood smear showing different forms of plasmodium vivax

(A) ring form; (B) gametocyte; (C) schizont



P. malariae – band form Fig: 3

Thin blood smear showing ring form of plasmodium malariae (band form)

- Gametocyte of P.falciparum → sickle-shaped
- Gametocytes of other 3 species → spherical

## II. Quantitative buffy coat:

- Acridine orange stain is used to detect nucleic acid of parasite in infected RBCs

## III. Antigen detection

Antigen detection

| HRP-2 (Histidine rich protein – 2) | LDH (lactate dehydrogenase) |
|---|---|
| • $HRP_2$ – present only in P. falciparum | • Present in all 4 species |
| • Not used for prognosis | • LDH levels fall with treatment |
| | • Therefore, used for prognosis |

# BABESIA

## Babesia 00:00:12

- It is an intra-erythrocytic protozoan of cattle and sheep
- It causes tick-borne malaria like illness
- Rarely infects humans (Causes opportunistic infections)
- Two important species:
  - B. microti: small species of Babesia
  - B. divergens: big species of Babesia
  - Host: Nymph stage of deer tick (Ixodes scapularis)
  - Definitive host: Ioxdes scapularis
  - Intermediate Host: Man
  - Man as intermediate host:
    - Babesia
    - Malaria
    - Toxoplasma
    - T. solium

### Mode of infection:

Most common – Bite of Tick

- The sporozoites are injected into the blood
- Sometimes infection can occur through blood transfusion
- Asexual cycle occurs in man

↓

Sporozoites injected into the blood,

Enter the RBCs and change into Trophozoites

↓

Multiply asexually by budding

↓

2/4 pear shaped ring forms are formed – "Maltese cross appearance"

↓

They feed on the Hb (no pigment)

- Maltese cross appearance is also seen in Cryptococcus neoformans
- Some of the asexual forms change into gametocytes
- Gametocytes are picked up by tick during blood meal

↓

Reach the intestine, where the gametocytes multiply sexually

↓

Some reach the salivary glands where they change into sporozoites.

## Pathogenesis and clinical features 00:07:00

- IP: 1–6 weeks
- Warm months
- Mild B. microti infection:
  - Fever

  - Fatigue
  - Weakness
  - malaise
- Severe Babesia microti infection:
  - Chills
  - Headache
  - Arthralgia
  - Myalgia
  - Dry cough
  - Nausea
- Most severe disease:
  - DIC
  - ARDS
  - CHF
  - Renal failure
  - Splenic infarcts and rupture

## Risk factors for severe and most severe disease:

1. Splenectomy
2. HIV
3. M>F
4. >50 years of Age
5. Malignancy
6. Immunocompromised state

- The disease is not yet reported in India
- It is endemic in
  - North eastern states – US

  - South East Massachusetts
- Occasional cases reported from Europe

## Diagnosis 00:10:08

i. Microscopy:

- Giemsa-stained peripheral blood smear-Maltese crosse appearance

ii. Serology:

- IFA test for B. microti

  IFA - confirms the diagnosis

  IgM titres of ≥ 1:64

  IgG titres of ≥ 1:1024

- Ab to B. microti do not cross react with other species of genus Babesia

iii. Molecular methods:

- PCR – Target 18s rRNA gene

iv. Animal inoculation:

- Blood of patient is injected into peritoneal cavity of golden hamsters
- After 2-4 weeks

  ↓

  Animal blood should be collected and observed weekly for 6 weeks to see the parasite

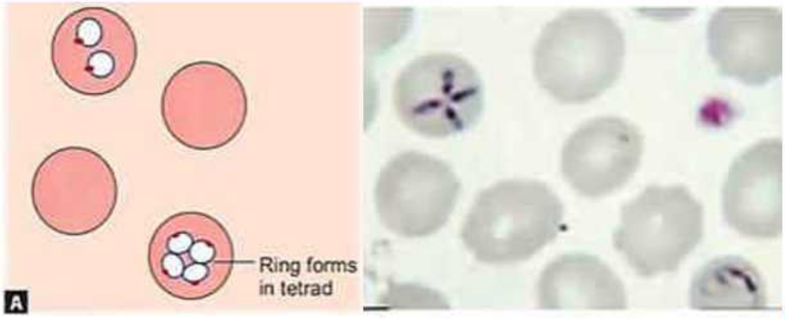


Fig: 1

## Treatment 00:13:32

| Treatment | Babesia |
|---|---|
| For mild Babesia microti illness: | The recommended regimen is oral atovaquone plus azithromycin for 7-10 days |
| For severe Babesia microti illness: | Intravascular (IV) clindamycin plus oral quinine should be given for 7-10 days and blood transfusion to be considered if required |
| • Other Babesia infection like Babesia divergens and Babesia duncani: | Immediate complete RBC exchange transfusion is recommended followed by IV clindamycin plus oral quinine for 7-10 days |

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# TOXOPLASMA GONDII

## Toxoplasma Gondii 00:00:19

- Non motile protozoan
- Definitive host – cat: Enteric cycle
- Intermediate host – Mouse: exocentric cycle
- Man – accidental host
- Intermediate host has tissue cyst
  - These has bradyzoites – slowly multiplying forms
- Cat gets infected by eating dead mouse containing tissue cyst

↓

Tissue cyst releases sporozoites

↓

Infect intestinal epithelial cells of cat

↓

Mature into micro and macrogametocytes

↓

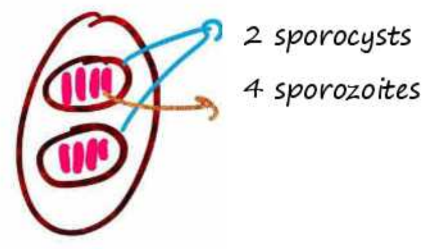
Fertilization

↓

Zygote

↓

Oocyst.



- Oocyst is excreted in faeces of cat
  - freshly passed oocyst is not infective to intermediate host
- Oocyst undergoes development in the soil to become infective (2-3 days)

↓

Infected oocyst is eaten by the mouse

↓

Oocyst releases the sporozoites and these sporozoites penetrate intestine and enter bloodstream

↓

In blood – they change into tachyzoites (rapidly multiplying forms)

↓

With time, immune response is mounted against tachyzoites

↓

They disappear from the blood and enter tissues Where they form bradyzoites

## How humans are infected? 00:10:16

1.by eating infective oocyst with food/water

2.by eating tissue cyst present in meat of animal

3.blood transfusion ] Tachyzoites are infective form

4.transplacental

## Clinical features 00:11:41

A. In immunocompetent people:

1.mostly asymptomatic

2.some cases – IMN – like syndrome

note: when man becomes intermediate host – tissue cyst forms in brain and myocardium

B.In immunocompromised people:

3.tissue cyst in brain can reactivate

4.congenital toxoplasmosis: Chorioretinitis is most common feature

Note:

- if mother has IgG antibodies – No risk to baby

↓

Presence of IgG in the mother indicates that the mother was exposed to the parasite and recovered

- if mother has IgM, it indicates that the mother is exposed to the parasite and infection is active

## Transplacental transmission of Toxoplasma gondii

00:15:28

Transplacental transmission of Toxoplasma gondii

infection in

| 1st Trimester | 3rd Trimester |
|---|---|
| ↓ | ↓ |
| Lowest incidence (15%) of transplacental infection | highest incidence (65%) of transplacental infection |
| But | But |
| "Most severe disease in neonate" | "Infant is usually asymptomatic at birth" |

## Toxoplasma gondii 00:00:19

i. Intraperitoneal inoculation of blood of patient in mouse

ii. Sabin Feldman dye test:

- Gold standard
- Needs live tachyzoites
- Detects IgG

iii. IgM detection by ELISA

iv. IgG Avidity ELISA – Tells how old/chronic the infection is

(Increased avidity – more chronic the infection)

v. For congenital toxoplasmosis:

- IgM ELISA
- IgA ELISA (increased sensitivity)

vi. PCR

## Treatment 00:19:19

- DOC – Sulfadiazine + Pyrimethamine
- DOC in pregnancy – Spiramycin

# SMALL INTESTINAL NEMATODES

## Helminths 00:00:10

- Classification
  - Nemathelminths/Round worms/Nematodes
  - Platyhelminths/Flat worms

Nematodes – Have body cavity

- Intestinal Nematodes
- Tissue Nematodes

- This classification is based on where the adult worm lives

## Small Intestinal Nematodes 00:02:12

I. **Ascaris lumbricoides/Giant Roundworm:**

**EGG:**

- Egg is oval to round in shape
- It has rough surface
- The egg is bile stained
- Embryonated egg is the infective form
- Mode of infection: Ingestion of egg

Intake of embryonated egg

↓

Egg hatches in Intestine

↓

Larva Released

↓

Pierces the intestinal wall

↓

Enters venous circulation

↓

Reaches the right side of heart

↓

Exists the pulmonary artery

↓

Pierces the pulmonary capillary

↓

Lung parenchyma

↓

Ascends up & reaches throat

↓

& then swallowed

↓

Larva therefore reaches Intestine (2nd time entry of larva into intestine)

↓

Adults are formed (male & female)

↓

Fertilization occurs

↓

Female worms are oviparous & lays eggs

↓

Eggs is passed out in faeces

Is freshly passed egg infective? – No

- Because: freshly passed egg needs to develop in warm, moist soil for few days & then it becomes the **Embryonated egg.**

Clinical features due to

**Adults**

- Intestinal symptoms like – Abdominal pain, diarrhea
- Intestinal obstruction; perforation can occur.
- If worms enter CBD – Obstructive jaundice
- Malabsorption is never caused by Ascaris ∵ These are luminal worms

**Larva**

- Larva is present in lungs.
- Loeffler's pneumonia is caused:
  - It refers to pneumonia with an eosinophilic exudate in response to larva antigens

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- Diagnosis:
  i. Sample: faeces examination
    - On macroscopic examination – Adults are seen
    - On microscopic examination – eggs are seen

In faecal sample – larva are never seen

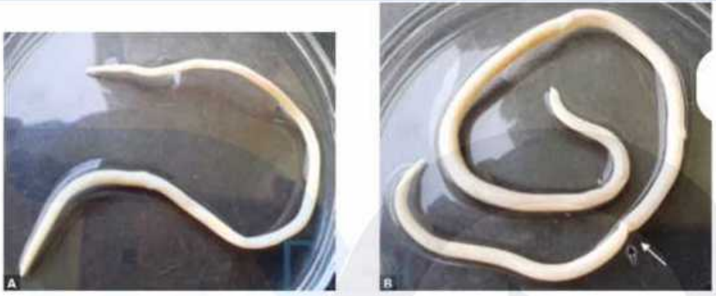
ii. Sputum examination – for larva

- Treatment:

  Mebendazole

  Pyrantel pamoate

  Epidemiology: present in tropical countries



ADULT ASCARIS WORMS A-MALE; B-FEMALE Fig: 1

## II. Hookworms

Hookworms

include

Ankylostoma duodenale
(old world hookworm)

Necator americanus
(New world hookworm)

- Mode of infection – skin penetration
- Infective form: filariform larvae
- Hookworms cause iron deficiency anaemia (IDA)
- Ankylostoma duodenale sucks 0.3 ml blood/worm/day
- Necator americanus sucks 0.03ml blood/worm/day

Filariform larva
↓
Penetrates skin of a bare-foot person
↓
Enters the venous circulation
↓
Right side of heart
↓
Exists pulmonary artery
↓
Pierces pulmonary capillary
↓
Reaches lung parenchyma
↓
Ascends up-reaches throat
↓
It is then swallowed
↓
Reaches small intestine for the first time
↓
Adults form (Males & Females)
↓
Fertilization occurs
↓
Female in oviparous – lays eggs
↓
Eggs passed out in faeces

- Egg – Is round to oval in shape
    - Smooth surface
    - Non-Bile stained

- Egg has segmented ovum, each segment is known as blastomere

Hook worm egg is opposite to Ascaris Egg

- Question: Is freshly passed egg Infective? Answer: No
  Because freshly passed egg develops in soil & forms
  **Rhabditiform larva** (Non-infectious, feeding larva)
  ↓ Transforms to
  **Filariform larva** (Infective & non-feeding larva)

## Clinical features of Hookworm infection are due to:

| Adults | Larva |
|---|---|
| • Live in small intestine<br>• Cause Abdominal pain, Diarrhea<br>• Iron deficiency anemia because of :<br>a. Adult worms feed on the blood present in capillaries of microvilli<br>b. Oozing of blood at the site because of production of an anticoagulant by the worm. | • Larva is present in the lungs<br>• It causes Loeffler's pneumonia.<br>• Ground Itch/skin rash at the site of skin penetration. It is a pruritic papule or vesicle. |

### Diagnosis:

- Sample: Faeces

  1.Faeces examination:

    Macroscopy – for adult worm

    Microscopy – for eggs

  2.Sputum – to look for larvae

  3.Occult blood in stool

  4.Eosinophilia

### Treatment:

- Mebendazole
- Pyrantel pamoate

### Prevention:

- Proper sewage disposal
- Proper foot wear

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### III. Strongyloides stercoralis

- Mode of infection: skin penetration
- Infective form: Filariform larva

Filariform larva penetrates
skin
↓
Enters venous circulation
↓
Reaches right side of heart
↓
Exists the pulmonary artery

↓

Pierce pulmonary capillary

↓

Reach lung parenchyma

↓

Ascend up

↓

Reaches throat

↓

Then swallowed

↓

Reaches small intestine

↓

Adults form (male & female)

↓

Female worm is Ovo-viviparous

↓

Female lays egg, which immediately hatches into larva - Rhabditiform larva in small intestine

Rhabditiform larva - fate:

It Can be passed out in faeces

↓

Reaches soil

↓

Transforms into Filariform larva

It can change into filariform larva in intestine itself

↓

Auto infection can occur

- Strongyloides stercoralis – is the only worm in Helminthology which is OVOVIVIPAROUS
- In immunocompromised patients, specially HIV patients,

  Disseminated strongyloidiasis can occur

  ↓

  Characterized by:
- Presence of larva in different organs/tissues

  Eg: Brain, kidney etc.
- In immunocompromised patients, integrity of gut mucosa is jeopardized by the penetrating larvae
- Due to this, enteric bacteria (Bacteroides fragilis, E. coli) enter the bloodstream and cause septicemia which can lead to death
- It is a free-living as well as parasitic worm
- Female worm is parthenogenic (Female worm can lay eggs without fertilization by male worm)
- Stool sample – only have larva: either Rhabditiform/Filariform, but never eggs

## Diagnosis:

- Specimen: Stool/faeces:
  - Only larvae are seen, not eggs
- Concentration method for stool specimen is **Bearmann's technique**
- Culture of larvae by Harda-mori culture

## Drug of choice:

- Ivermectin

NOTE: It is also the DOC for Onchocerciasis

- Alternative Drug: Thiabendazole

# LARVA MIGRANS AND LARGE INTESTINAL NEMATODES

## Larva migrans: 00:00:08

- 2 Types:

| Cutaneous larva migrans/Creeping eruption | Viceral larva migrans |
|---|---|
| Most common cause → Ancylostoma brasiliensis | Most common cause → Toxocara canis |
| Animal hook worm | Animal round worm |
| Infective form: Larva<br>Mode of infection: Skin penetration<br>↓<br>Larva migrates in skin | Egg<br>Ingestion of egg<br>↓<br>Hatches<br>↓<br>Releases larva<br>↓<br>Reaches viscera<br>More eosinophilia<br>More dangerous |

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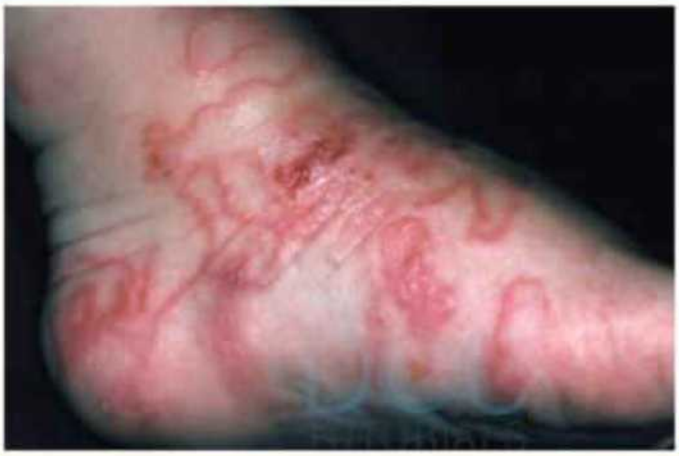

Fig: 1

### Ocular larva migrans:

- It is a sub-variety of visceral larva migrans
- Retinoblastoma is an important different diagnosis for ocular larva migrans

## Large intestinal nematodes 00:05:45

- Important ones are:

  Enterobius vermicularis

  Trichuris trichiura

## Enterobius vermicularis 00:06:53

- Also known as Pinworm/Seatworm/Threadworm
- Disease caused is oxyuriasis
- Infective form: EGG

### Egg:

- Plano-convex shape
- Non-bile stained
- Tadpole like larva inside

### Mode of infection:

- Ingestion of egg

  ↓
- Larva is released in small intestine

  ↓
- Adults form in small intestine

  ↓

- Adults reach large intestine where fertilization occurs

↓

- Female worm comes out of anus between 10pm to 4am to lay eggs in the perianal region

↓

- Within 6 hours eggs become embryonated
- These eggs are not passed out in faces & are stuck onto perianal region
- The patient presents with perianal pruritus
- Auto-infection can occur because of intake of eggs after scratching the perianal region and putting finger into the mouth
- Rarely appendicitis can occur

### Diagnosis:

- Eggs can be shown by:
  - Scotch tape method
  - NIH swab (National institute of health, USA)

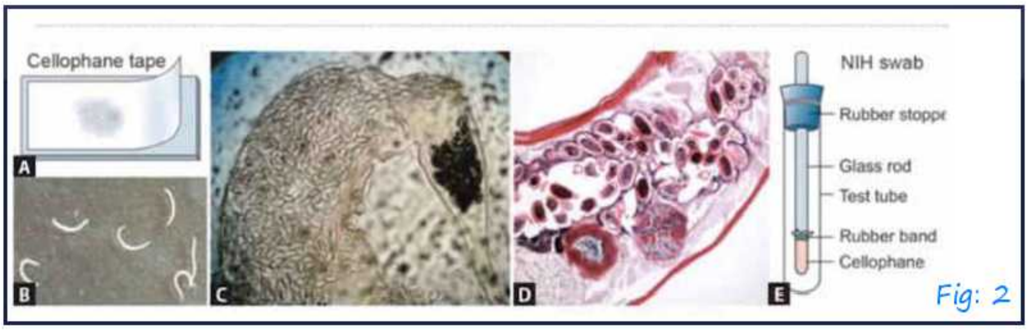
Cellophane tape
A
B
C
D
NIH swab
Rubber stoppe
Glass rod
Test tube
Rubber band
Cellophane
E

Fig: 2

FIGURE 2-Enterobius vermicularis (A) cellophane tape; (B) adult worm (actual size); (C) adult female worm containing numerous eggs; (D) longitudinal section of an adult female worm shows many planoconvex eggs; (E) NIH swab method

- No Loeffler's pneumonia
- Treatment: Mebendazole & Pyrantel pamoate
- These two drugs kill adult worms in colon but not eggs so re-treatment in 12weeks is needed
- Sometimes, small whitish worms may be present in faeces or found stuck to anus in diapered children

## Trichuris trichiura 00:19:13

- Infective form: egg
  - Barrel shaped
  - Both ends have mucous plugs
  - Bile stained
- Mode of infection -Ingestion of egg

↓

Egg hatches to produce larva in large intestine

↓

Adults are formed

↓

Fertilization occurs

↓

Female lays eggs

↓

Eggs are passed out in facees

- Question-Are freshly passed eggs Infective? Answer-NO

  (Because egg needs to develop in soil to become embryonated egg)

## Clinical features:

- Abdominal pain, diarrhea
- Rarely appendicitis
- On sigmoidoscopy the appearance is described as 'coconut cake rectum'

## Diagnosis:

- Egg demonstration in faeces

## Treatment:

- Mebendazole
- Pyrantel pamoate
- No Loeffler's pneumonia in this infection

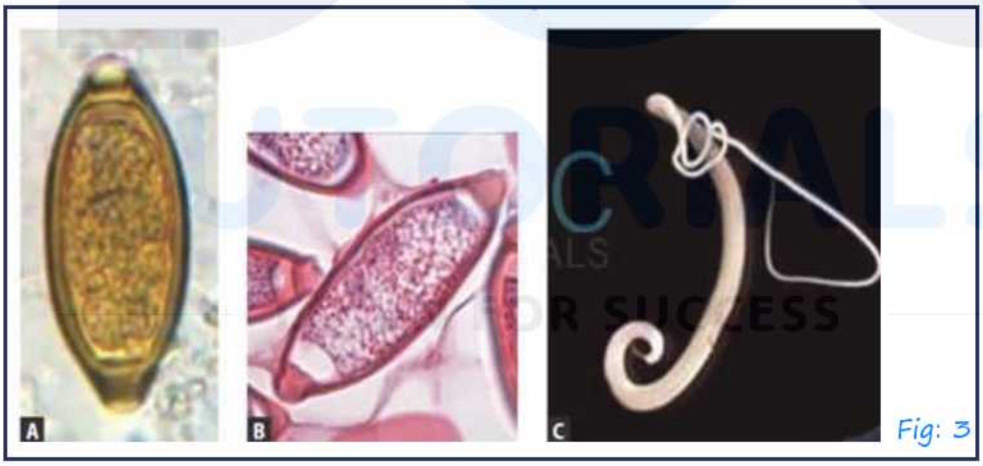

FIGURE 3: A-Egg in iodine mount

B-Egg in H & E stain

C-Adult female

- Also known as whipworm
- No autoinfection

# TISSUE NEMATODES PART I

## Tissue nematodes 00:00:10

- Also known as somatic nematodes
- Filarial nematodes: which cause lymphatic filariasis

  Lymphatic filariasis

  - Washeteria bannerette (95% cases in India)
  - Brugia malayi (5% cases in India)

- Brugia timori: is found in Indonesia and not India

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**For both W. bancrofti and B. malayi: -**

- Life cycle is same
- Definitive host is man
- Intermediate host is mosquito
- In W. bancrofti, intermediate host is culex fatigans
- In B. malayi, intermediate host is mansonia annulifra
- The inpective form for humans is 3rd stage filariform larva

**Life cycle:**

Mosquito bites human

↓ injects the infective forms

Larva enters subcultures tissue through bite wound

↓

Reaches the regional LNs

↓

Reaches the lymphatic vessels

↓

Here: larva forms the adults fertilization occurs

↓

Female: produces larva known as (Viviparous)
Microfilariae

↓

Enter the circulation
Microfilaria are detected in the peripheral blood smear
for diagnosis.

- Microfilaria are not responsible for the disease
- Disease that is, lymphatic filariais is because of adults present in the lymphatic vessels

All tissue nematocles are viviparous

Adults in the lymphatic vessels

↓

These adult worms incite inflammation of lymphatic
vessels
Lymphangitis

↓

Repeated attacks of lymphangitis
Leads to lymphatic obstruction

↓

Lymphangio varix

↓

These swollen lymphatie vessels can rupture and lymph is released

↓

LYMPH is a nutritious fluid and it causes hypertrophy and hyperplasia

Lymph enters the potential carity causing

- CHYLOTHORAX – lymph accumulates in the pleural cavity
- CHYLOUS ASCITES – lymph accumulation in peritoneal cavity
- CHYLURA – lymph excreted in urine
- CHYLOCELE – lymph accumulated in scrotal sac

- Chylocele is exclusively seen with W. banerofti
- Elephantiasis of legs: Irreversible edema of legs

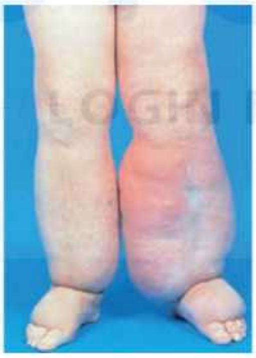


Fig: 1

- Elephantiasis is seen in edemic areas but not in tourists

  Most infection – are asymptomatic initially later develop – fever, lymphangitis

### Microfilariae

Microfilariae are seen in blood in the acute phase of disease and not in chronic phase of the disease

- This is because, in chronic phase – there in lymphatic obstruction – which does not allow microfilariae to enter blood circulation
- Tropical pulmonay eosinophilia (TPE) or occult filariasis or Weingarten syndrome
  - Microfilaria get filtered from blood and enter lungs where they are destroyed
    Hypersensitivity reaction develops against the laral antigens
  - There are no microfilariae present in peripheral blood- hence called occult filariasis

### Clinical features:

- Nocturnal paroxysmal cough
- Low grade fever
- Loss of weight
- M:F = 4:1, occurs in 3rd decade of life

### Diagnosis:

- Peripheral blood eosinophilia
- Increased Ig E in blood
- Chest x-ray: shows diffuse infiltrates
- Pulmonary function test: shows obstructive pattern

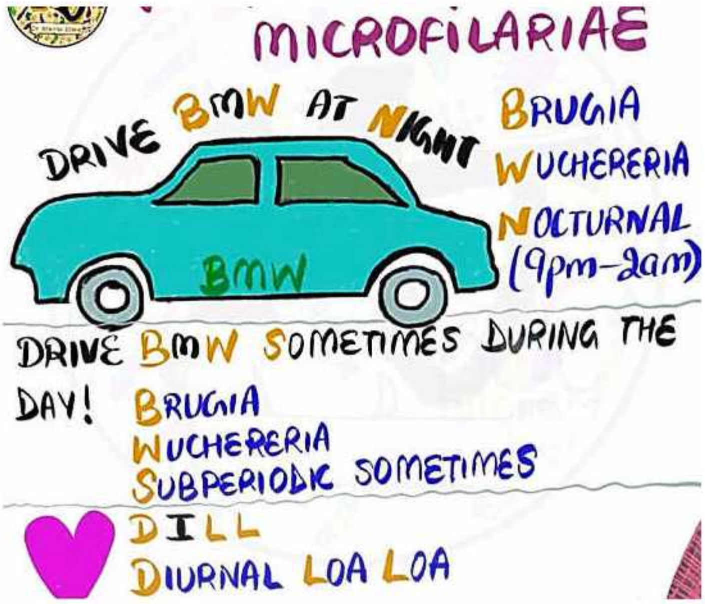

MICROFILARIAE
DRIVE BMW AT NIGHT
BRUGIA
WUCHERERIA
NOCTURNAL
(9pm-2am)
BMW
DRIVE BMW SOMETIMES DURING THE DAY!
BRUGIA
WUCHERERIA
SUBPERIODIC SOMETIMES
DILL
DIURNAL LOA LOA

Fig: 2

Sometimes, microfilariae may get filtered in sites other than lungs

Example: liver
Spleen
lymph nodes

This leads to hepatosplenomegaly and lymphadenopathy Meyer's kouwenaor syndrome

## Diagnosis

- Peripheral blood smear: for microfilariae blood sample is taken at night
- DEC provocation test
  - Diethylcarbamazine is given to patient which induces the microfilariae to enter the blood during day time
- Ag – detection by ELISA – though CDC at atlanta

## Drug of choice: DEC

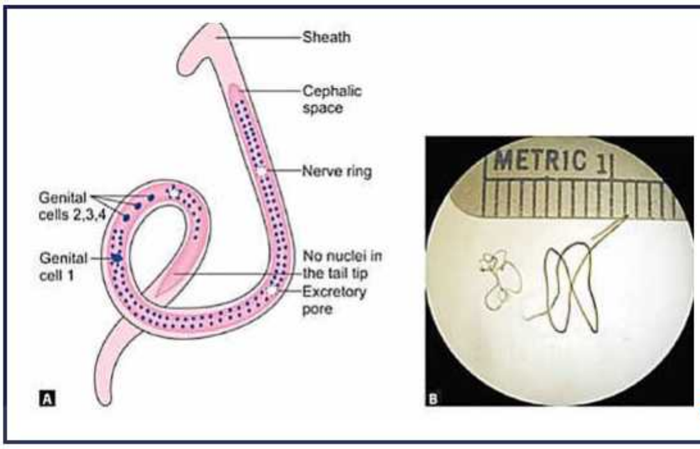

Sheath
Cephalic space
Nerve ring
Genital cells 2,3,4
Genital cell 1
No nuclei in the tail tip
Excretory pore
A
METRIC 1
B

Fig: 3

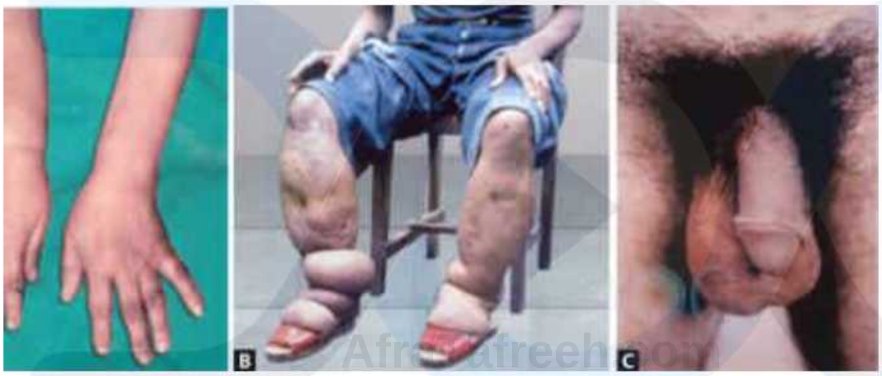


Fig: 4

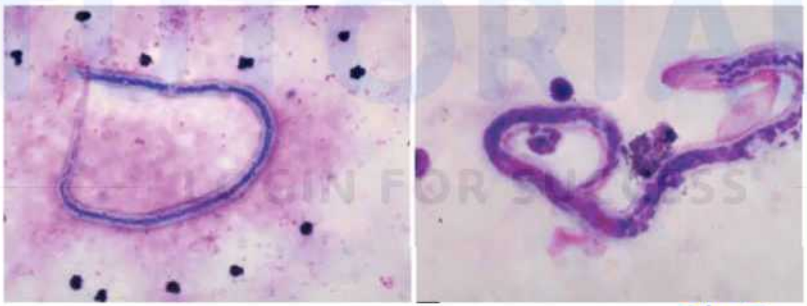
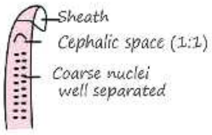
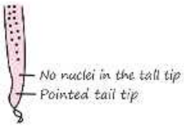
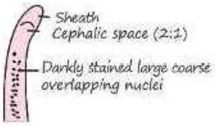
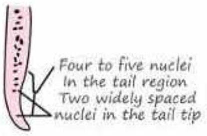
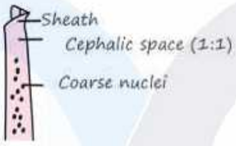
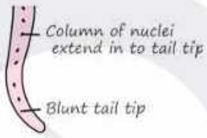
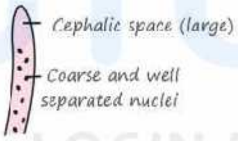
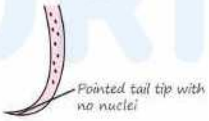
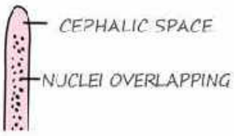
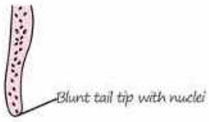
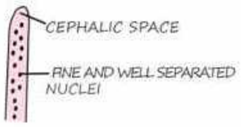
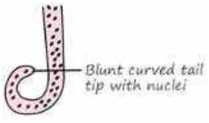
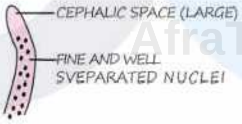
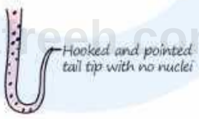


Fig: 5

| Microfilaria | Head end | Tail end | Features |
|---|---|---|---|
| Wuchereria bancrofti |  Sheath<br>Cephalic space (1:1)<br>Coarse nuclei well separated |  No nuclei in the tail tip<br>Pointed tail tip | A: 240-300 µm<br>B: Nocturnal |

| | | | |
|---|---|---|---|
| | | | C:<br>Sheathed<br>D: Blood |
| Brugia malayi |  Sheath<br>Cephalic space (2:1)<br>Darkly stained large coarse overlapping nuclei |  Four to five nuclei In the tail region<br>Two widely spaced nuclei in the tail tip | A: 220 μm<br>B: Diurnal<br>C:<br>Sheathed<br>D: Blood |
| Loa loa |  Sheath<br>Cephalic space (1:1)<br>Coarse nuclei |  Column of nuclei extend in to tail tip<br>Blunt tail tip | A: 250-<br>300 μm<br>B: Non periodic<br>C:<br>Sheathed<br>D: Blood |
| Onchocerca volvulus |  Cephalic space (large)<br>Coarse and well separated nuclei |  Pointed tail tip with no nuclei | A: 200-<br>300 μm<br>B: Non periodic<br>C:<br>Unsheated<br>D: Skin, eye |
| Mansonella perstans |  CEPHALIC SPACE<br>NUCLEI OVERLAPPING |  Blunt tail tip with nuclei | A: 190 -<br>200 μm |

| | | | |
|---|---|---|---|
| | | | B: Non periodic<br>C: Unsheathed<br>D: Blood |
| Mansonella Streptocerca |  CEPHALIC SPACE<br>FINE AND WELL SEPARATED NUCLEI |  Blunt curved tail tip with nuclei | A: 180-240 µm<br>B: Non periodic<br>C: Unsheathed<br>D: Skin |
| Mansonella ozzardi |  CEPHALIC SPACE (LARGE)<br>FINE AND WELL SVEPARATED NUCLEI |  Hooked and pointed tail tip with no nuclei | A: 200-230 µm<br>B: Non periodic<br>C: Unsheathed<br>D: Blood |

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# TISSUE NEMATODES PART II

## Dracunculus medinensis /Guinea worm /Medina Worm 00:00:10

- It is the longest nematode
- It has been eliminated from India

**Definitive host-Man**

- Intermediate host:
  - Cyclops (a fresh – water crustacean)
  - It is a copepod of 1-2mm size
- Cyclops has the larva
- Man gets infected by drinking contaminated water – water containing cyclops which has larva (L3 larva)

↓

Cyclops is digested

↓

Larva is released

↓

Larva penetrates the gut wall and enters retroperitoneal tissue, Here adult worms are formed

↓

Fertilization occurs

(Male worms die after fertilization)

↓

Female worms come out of that area of skin which repetitively comes in contact with water

↓

During this process, female worm lays 1000s of larvae, which are discharged into water

- This is the only worm, which exits through the skin
- In water, cyclops receives the larva

↓

This larva develops further in cyclops

↓

It becomes the L3 larva

### Clinical features:

- Skin blister (at the site, where female worm exits the skin)
  - This blister breaks and forms ULCER
  - This ulcer can get secondary bacterial infection

### Diagnosis:

- Using a match stick, the worms coming out of the skin are rolled out
- Peripheral blood eosinophilia
- Detection of $L_1$ larvae
- X-ray of retroperitoneal tissue – shows dead, calcified worms

## Treatment:

- No anti-helminthic drug
- Topical antibiotics for 2° bacterial infection
- Analgesics

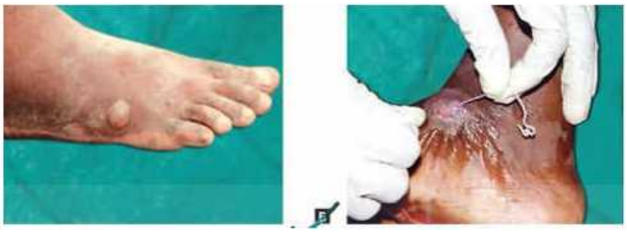

Fig: 1

Real image of (Left) blister formed in dracunculiasis; (Right) Dracunculus medinensis (adult female worm) emerging from the blister

# TISSUE NEMATODES PART III

## Onchocerca volvulus /Convoluted worm 00:00:10

- Whitish, opalescent worms, with posterior end curved. They are tiny worms with striations on the cuticle

Onchocerca volvulus Fig: 1

  - Disease: caused is Onchocerciasis / river blindness
  - Vector: Blackfly/Simulium
- This the most common cause of blindness in Africa
- Second most common cause of blindness worldwide
- Only worm, where microfilariae cause the disease
- The fly bites man and deposits infective larvae

↓

Larvae enter the bite wound

↓

They reach subcutaneous tissue where adults are formed

↓

Fertilization occurs

↓

Female worm releases microfilariae which enter the blood

## Onchocercomas:

- These are subcutaneous nodules containing the adult worms surrounded by fibroblastic reaction
- Size – varies from few mm to 10 cm
- These form at sites, where bones are superficial
  Example: scapula, scalp, ribs, Iliac crest

## Diagnosis:

- Skin-snip technique: to look for microfilariae
  - Taken from areas of maximum density of microfilariae
    example: Iliac crest
- Biopsy of oncocercomas to look for adults

## Treatment:

DOC: Ivermectin.

# TISSUE NEMATODES PART IV

## Loa loa / African eye worm / Eyeworm 00:00:10

- Adult worms crawl across the subconjunctival area
- Disease: Calabar swelling /loiasis
- Calabar swelling: It is a hypersensitivity reaction, localized, transient, non-erythematous subcutaneous edema
- Definitive host: Man
- Intermediate host: Mango fly/Chrysops /Deer fly
- The fly bites humans and deposits infective larvae

↓

Larvae enter the bite wound

↓

Enter the subcutaneous tissue and form adult worms

↓

Fertilization occurs

↓

Female releases microfilariae into blood

### Diagnosis:

- Detection of microfilariae in blood
- Detection of adults in subcutaneous tissue

### Treatment:

- Diethylcarbamazine

# TISSUE NEMATODES PART V

## Trichinella spiralis 00:00:10

- Causes Trichinellosis/Trichinosis
- Reservoir & most important host – pigs
- In humans: It is a zoonotic disease.

  We acquire it from domestic pigs or other carnivores
- 11 species are present in the genus Trichinella
  - Important species for humans – T. spiralis
- This infection is prevalent in pork eating countries, more common in temperature zones than tropical zones.

  Ex: North America, South America, Europe
- Also, meat of horses and wild bears is also implicated in this disease.

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**In India:** Animal infection is reported.

Human infection is uncommon.

- In 2010, there was an outbreak in Uttarakhand – 18 people were affected due to eating the meat of wild bear called kachmoli
- 1st case in india – Punjab

**Adult worms:**

- Female – 1.5 mm (Male)
- Male – 3 mm (Female)

- Smallest nematode
- It is thread-like

## Life Cycle 00:05:10

i. Pig – most important host and reservoir

ii. Animals like
Rats
Horses
– Other hosts

iii. Transmission occurs from 1 flesh eating animal to another

iv. Common cycles: Pig to Pig
Rat to Pig
pig to Rat

v. Man – Accidental host (dead end)

- Infective form – $L_1$ Larva
- Mode of infection – Ingestion of raw/uncooked food like pork or other animal meat containing $L_1$ Larva

### I. Intestinal phase:

Larva ingested with the meat
↓
Release of larva from the flesh by enzymes present in the stomach
↓
Larva goes to small intestinal mucosa where 4 moltings occur to form adult worms in 2-3 days.

- Female worms – lay $L_1$ larva

- Female worms – Die after mating with male worms
- After 5 days of fertilization, the first stage larva begins to be laid.

### II. Migration phase:

- L1 Larva penetrates the intestinal wall

and

Carried to skeletal muscles via blood and lymphatic vessels.

↓

"Encystment in skeletal muscles"

### III. Encystment phase:

Encystment: $L_1$ larvae enter skeletal muscles cells and behave as obligate intracellular anaerobic parasites

- In 20 days – muscle cell becomes modified to form Nurse cell.
- Nurse cell is surrounded by blood vessels.
- Only skeletal muscles are involved, not the cardiac or smooth muscles.

## Organization 00:13:42

- After some years, the nurse cell-larva complex undergoes changes.

Eg: Fibrosis
Degeneration
Calcification

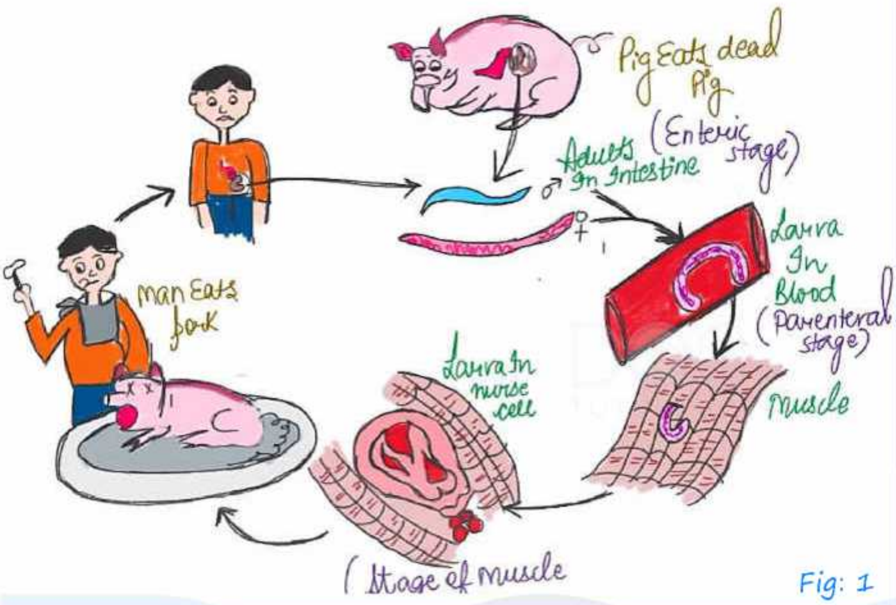
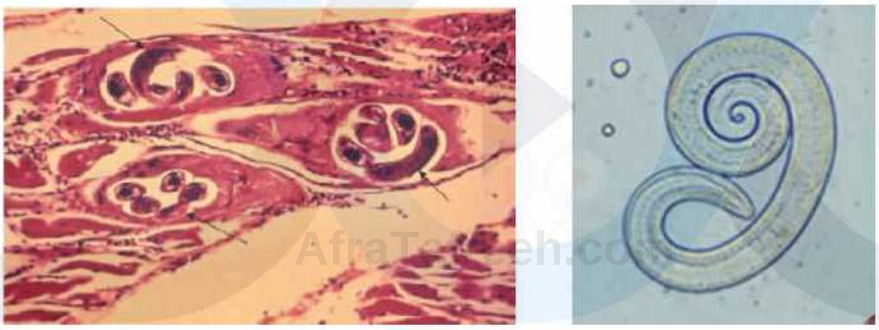

Pig Eats dead Pig
Adults In Intestine (Enteric stage)
♂
♀
Larva In Blood (Parenteral stage)
Muscle
Larva In nurse cell
( Stage of muscle
man Eats pork

Fig: 1



Left: Trichinella cysts within human muscle tissue (hemotoxylin and eosin stain) Right: Larvae of Trichinella liberated from bear meat (microscopic view)

Fig: 2

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## Clinical Features 00:17:02

**I. Intestinal phase:**

- Most infections are asymptomatic
- Symptoms occur during the 1st week
- Watery diarrhea
- Abdominal pain, nausea & vomiting

## II. Migration phase/Parenteral phase:

- Symptoms appear in 2nd week after infection
- Hypersensitivity reaction – fever, eosinophilia.
- Periorbital edema ⎤ Common
- Facial edema ⎦
- Retinal hemorrhage
- Subconjunctival hemorrhage
- Hemorrhage in nail bed (splinter hemorrhage)
- Maculopapular rash
- Migration can occur to heart – Myocarditis ⎤ Become severe in 4-8 weeks
  CNS – Encephalitis
  Lungs – Pneumonia ⎦
- Myocarditis is transient as encystment does not occur in the cardiac muscle

## III. Encystment phase:

1. Clinical features occur 2-3 wks after infection
2. Myositis
   Myalgia
   Muscle weakness
   Muscle edema
3. Most common involved muscles: Extraocular muscles; followed by
   ↓
   Biceps

Other muscles – Jaws, neck, lower back and diaphragm

## Lab Diagnosis 00:23:48

### I. Demonstration of larvae on muscle biopsy:

- Sample – muscle biopsy taken from deltoid insertion (at least 1 gm)
  - Demonstration is done by H&E stain
  - Enzymatic digestion of sample is done before staining

### II. Serology:

- Specific antibodies can be detected by:
  i. ELISA
  ii. CIEP
- Becomes positive after 2 weeks of infection

### III. Bachman intradermal test:

- Becomes positive 2-3 wks after infection.
  - Intradermal injection of bachman antigen (prepared from Trichinella larvae got from rabbit muscle)

    ↓

    An immediate small induration area surrounded by edema of 5cm in diameter in 15-20 minutes
  - Remains positive throughout life

### IV. Animal inoculation test:

- Rats are fed with the muscle tissue of suspected patients.

↓ after appropriate time

Rats are examined for the presence of larvae in diaphragm

### V. Other tests:

a. Eosinophilia – >90% of asymptomatic patients.
   - Levels peak at 2–5 wks after infection, persists till 4–8 weeks.

b. Increased WBC count

c. Increases muscle enzymes (increases creatinine phosphokinase)

d. X – ray is done to see calcified muscle cyst

## Treatment 00:30:23

**Treatment**

| **Mild infection** | **Moderate infection** | **Severe infection:** |
|---|---|---|
| 1. Antipyretics<br>2. Bed rest<br>3. Analgesics | i. Mebendazole<br>ii. Albendazole<br>↓<br>Act on adult worms present in the intestine | Steroids for<br>a. Severe myositis<br>b. Myocarditis |

# INTRODUCTION TO CESTODES

## Cestodes/Tapeworms 00:00:10

- They are platyhelminths/Flat worms
  1. Taenia saginata/Beef tapeworm/Unarmed tapeworm
  2. Taenia solium/Pork tapeworm/Armed tapeworm
  3. Echinococcus granulosus/Dog tapeworm
  4. Hymenolepis nana/Dwarf tapeworm
  5. Diphyllobothrium latum/Fish tapeworm

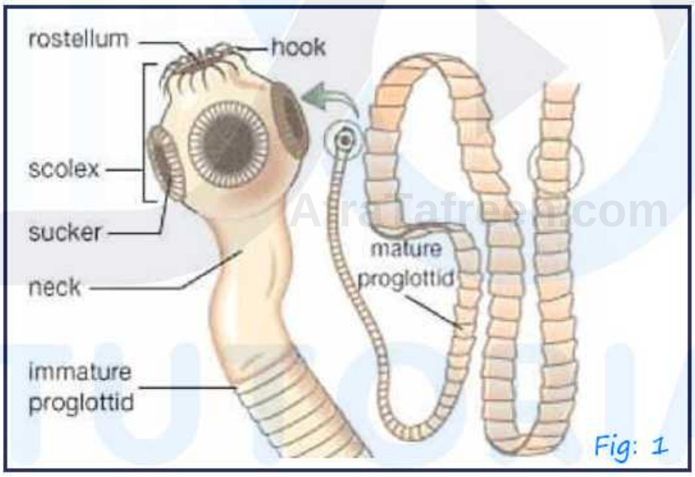
rostellum
hook
scolex
sucker
neck
immature proglottid
mature proglottid

Fig: 1

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- All cestodes produce embryonated eggs
- Eggs of most cestodes have an embryo having 6 hooklets (Known as hexacanth embryo/oncosphere)
  Exception: Egg of Diphyllobothrium latum which has a ciliated larva & an operculum
- Eggs of all cestodes are bile stained except the egg of H. nana – Non bile stained

- All cestodes need a definitive host & an intermediate host (IH) to complete their life cycle. Except: H. nana (Man is the only host)
- D. latum: Longest cestode (15metres)

## Taenia saginata 00:08:32

- Also known as: Beef tapeworm/Unarmed tapeworm
- Definitive host: Man
- Intermediate host: Bovines
- Intermediate host have the larvae in the tissues
- Therefore, bovines have 'cysticercosis bovis' larva
- Man gets infected by consumption of undercooked beef – which has the larva – Intestinal taeniasis

↓

Larva enter the intestine & form adults

↓

Fertilization occurs

↓

Female lays eggs

↓

Eggs are passed out in faeces + Proglottids are passed out

### Clinical features:

- Most cases-no symptoms/asymptomatic
- There may be mild symptoms in some like abdominal pain diarrhoea

## Diagnosis:

- Faeces examination for eggs & proglottids (motile)
- Scotch tape technique to see the proglottids

## In bovines:

- Eggs are passed out in faeces of infected person

↓

Eggs reach the soil

↓

These eggs are ingested by cattle while grazing

↓

Egg hatches in the intestine

↓

Hexacanth embryo released

↓

The embryo, with the help of hooklets, penetrates the gut wall

↓

Enters blood

↓

Matures into larvae in the tissues

- Therefore, for the intermediate host, infective form is the egg and larvae are present in its tissues.

## Treatment:

- Praziquantel/Albendazole

## Taenia solium/Armed tapedworm/Pork tapeworm

00:17:40

- Definitive host: Man
- Intermediate host: Pig (Has larva known as cysticercus cellulosae)
- Man gets infected by consumption of undercooked food which has larva

Intestinal taeniasis:

↓

Larvae form adults in the intestine

↓

Fertilization

↓

Female worm lays eggs

↓

Eggs passed out in faces

↓

Proglottids too passed out sometimes

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- Most cases are asymptomatic
- Some may develop mild symptoms like abdominal pain and diarrhoea

### Diagnosis:

- Faecal examination for eggs and proglottids
- T. saginata eggs are acid-fast

### Treatment:

- Praziquantel
- Albendazole

### In pigs:

- When eges are ingested by the pigs

  ↓

  Larvae formation occurs in tissues of pig

- When man becomes both intermediate host & Definitive host

  ↓ Then

  Cysticercosis develops in man

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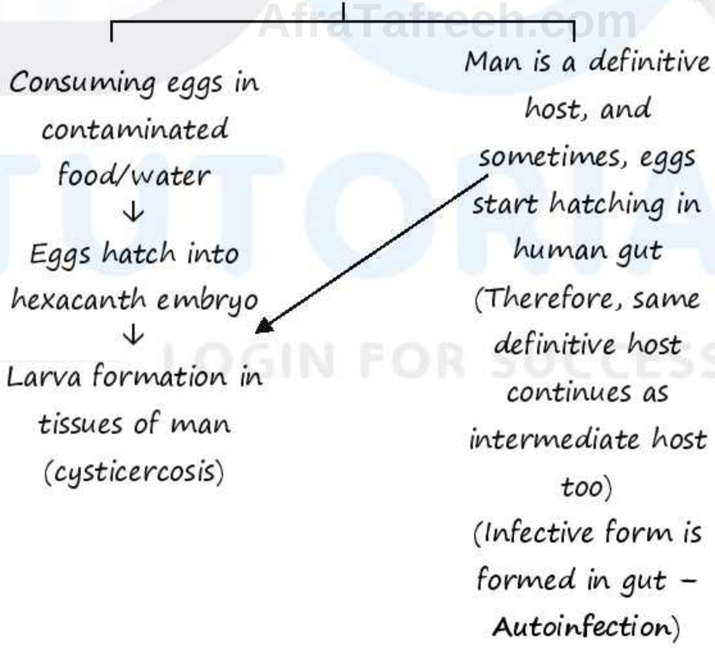
**Man becomes intermediate host by**



Consuming eggs in contaminated food/water
↓
Eggs hatch into hexacanth embryo
↓
Larva formation in tissues of man (cysticercosis)

Man is a definitive host, and sometimes, eggs start hatching in human gut (Therefore, same definitive host continues as intermediate host too)
(Infective form is formed in gut – **Autoinfection**)

i. Neurocysticersosis: Most common type of Cysticercosis
- Larva in CNS
- Most common symptom-seizures
- Most common site – Subarachnoid space
- 2nd common site: Brain parenchyma
- In India: Neurocysticersosis is the most common parasitic CNS infection.

Note: Most common CNS infection in India is Mycobacterium tuberculosis

ii. Subcutaneous cysticercosis:
- Asymptomatic or palpable nodules.

iii. Muscular cysticercosis:
- Muscular pain, weakness, pseudohypertrophy of muscles.

iv. Ocular cysticercosis:
- Involve sclera, lids, conjunctiva.
- Can lead to proptosis, loss of vision & diplopia

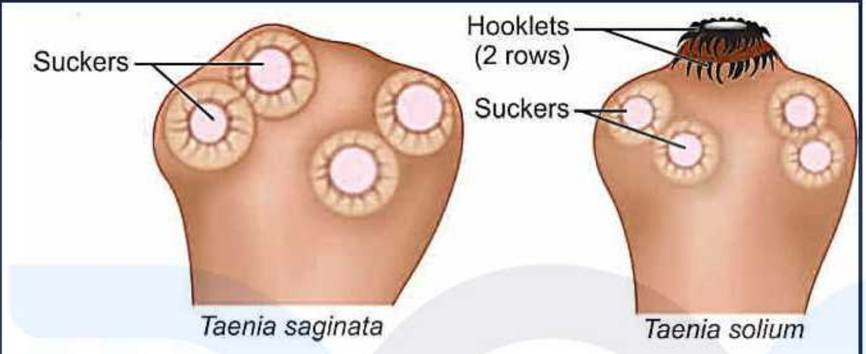
- In case of Cysticercosis in Man:
  - Infective form is egg
  - Man is both intermediate & definitive host

## Diagnosis of Cysticercosis:

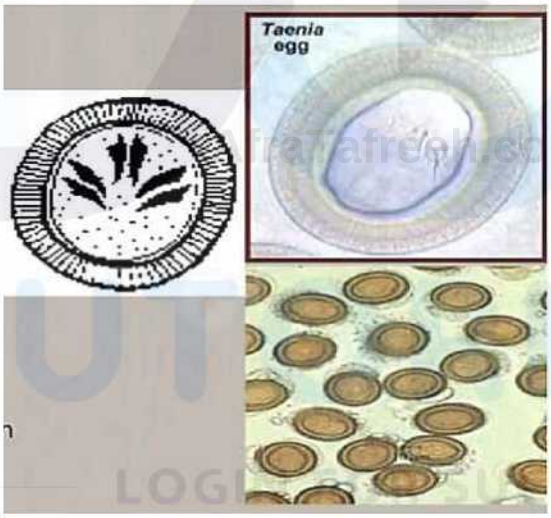
- Imaging techniques
- Antibody detection by ELISA

### Treatment: Praziquantel

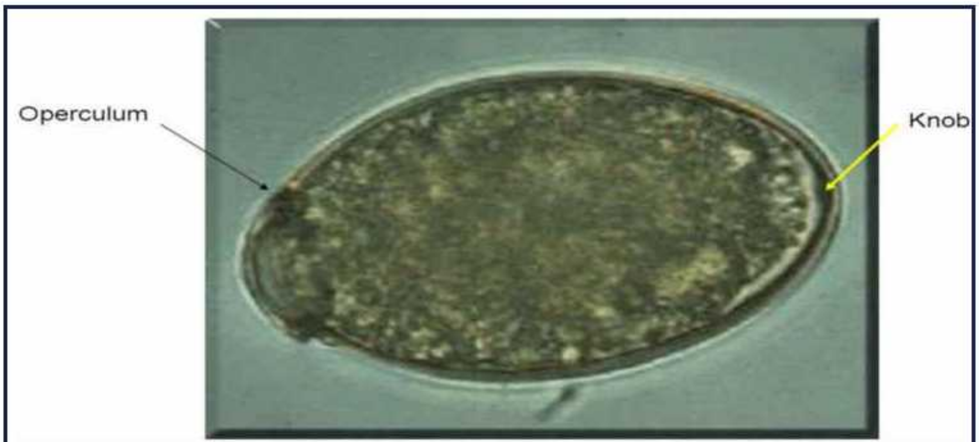
- Eggs of both T. solium & T. saginata are bile stained.
- Adults of both T. solium & T. saginata stay in jejunum & Ileum.
- Head of T. saginata is longer than T. solium


Suckers
Hooklets (2 rows)
Suckers
Taenia saginata
Taenia solium

Scolices of T. saginata and T. solium Fig: 2



Eggs of Taenia Fig: 3


Operculum
Knob

Egg of D.latum Fig: 4

# ECHINOCOCCUS GRANULOSUS

## Echinococcus granulosus 00:00:15

- Also known as dog tapeworm
- Definitive host: Dog
- Intermediate host: Sheep
- Accidental intermediate host: Man
- Intermediate host has the hydatid cyst (larva)

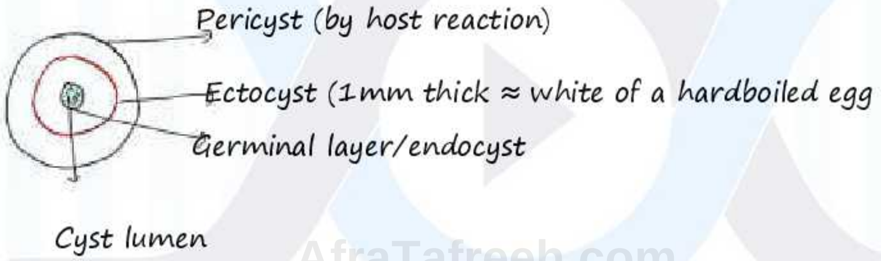
Dog eats sheep meat (which has larva)
↓
Larva reaches intestine of dog
↓
Adults form, fertilization occurs
↓
Female worm lays the eggs
↓
Eggs are excreted in the faeces of dog
↓
Eggs are eaten by sheep
↓
Hexacanth embryo is released
↓
Hooklets pierce the intestinal wall
↓
Larva is formed in the tissues of sheep

**For man-eggs can be the infective form**

Therefore, man becomes – accidental I.H

- Larvae form in man

- Liver – it is the most common site for deposition of larvae (hydatid cyst)
- Second common site – Lungs
- Other sites:
  - Spleen
  - Kidney
  - Brain
  - Heart
  - Rarely bones


Pericyst (by host reaction)
Ectocyst (1mm thick ≈ white of a hardboiled egg
Germinal layer/endocyst
Cyst lumen

- Pericyst contains → blood vessels and fibrous tissue
- Ectocyst → it is a acellular laminated layer
- Germinal layer/endocyst
  - It gives rise to protoscolices present in brood capsules
  - These are younger forms of the parasite
- Ectocyst – is tough, elastic and glycan-rich layer
- Cyst lumen has a highly allergic, watery fluid with low specific gravity
- This fluid has salts of sodium ($Na_3 Po_4$, NaCl etc.)
- $P^H$ of this fluid is 6.7

## Clinical features:

- Most cases are asymptomatic
- Symptoms are due to
  - Rupture of cyst-anaphylaxis
  - Pressure symptoms

    Example: recurrent attacks of cholecystitis
- Palpable mass per abdomen (Hepatomegaly)
- Portal hypertension
- Ascites

## Treatment:

- PAIR – Percutaneous Aspiration, Injection of scolicidal agent (example: absolute alcohol), Reaspiration)

## Diagnosis:

- Imaging
- Serology is the mainstay:

  Ab – detection by ELISA
- Casoni's test (obsolete now)
  - It is a skin test, based on type – I H/S reaction

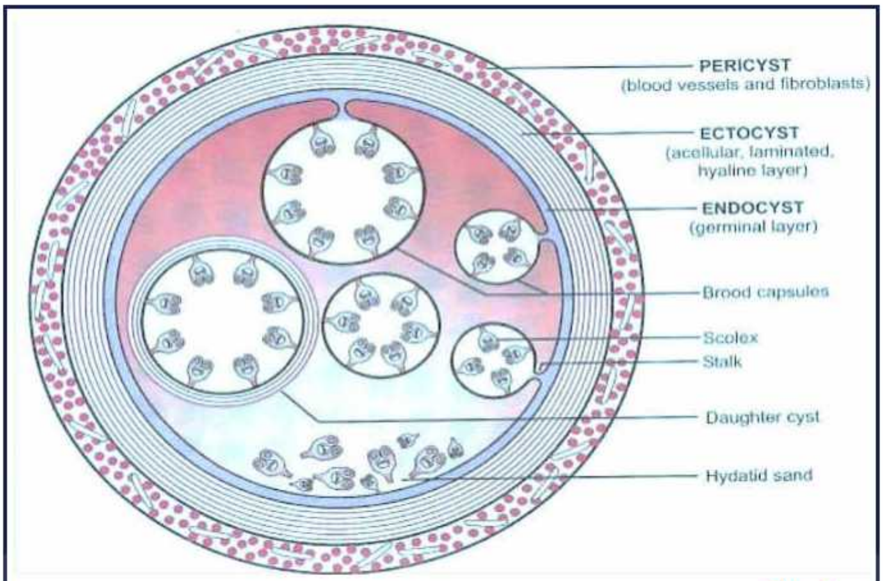

PERICYST
(blood vessels and fibroblasts)
ECTOCYST
(acellular, laminated, hyaline layer)
ENDOCYST
(germinal layer)
Brood capsules
Scolex
Stalk
Daughter cyst
Hydatid sand

Fig: 1

# HYMENOLEPIS NANA

## Hymenolepis nana /Dwarf tapeworm 00:00:10

- A cestode with non-bile-stained eggs
- Man – only host
- Infective form: egg
- Infection occurs by eating food/water contaminated with eggs

↓

Egg hatches in the intestine and releases oncospheres

↓

Onchosphere penetrates the intestinal
villi and develops into cysticercoid larva

↓

Cysticercoid larva migrates back to the lumen,
attaches to mucosa and then matures into adults
Adults ~2 cm long and are present in proximal ileum

↓

Fertilization occurs, female lays eggs

↓

Eggs can hatch in the intestine and hence can cause
autoinfection

### Clinical features:

- Abdominal pain, diarrhea

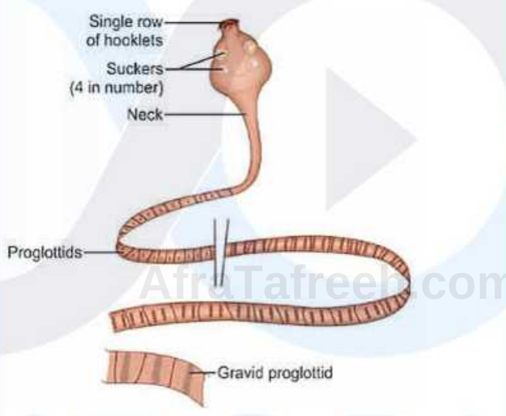
## Diagnosis:

- Presence of eggs in faeces can be demonstrated

  H. nana – Prominent in temperate and tropical regions

  - Overall, most common cestode infection
  - A thin membrane is present covering the eggs

  Scolex – has a single row of 20-30 hooklets (armed tapeworm)


Single row of hooklets
Suckers (4 in number)
Neck
Proglottids
Gravid proglottid

Adult worm of H. nana Fig: 1

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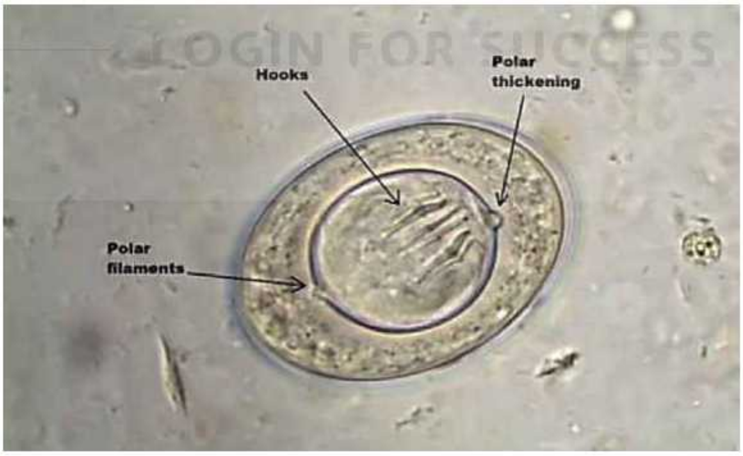
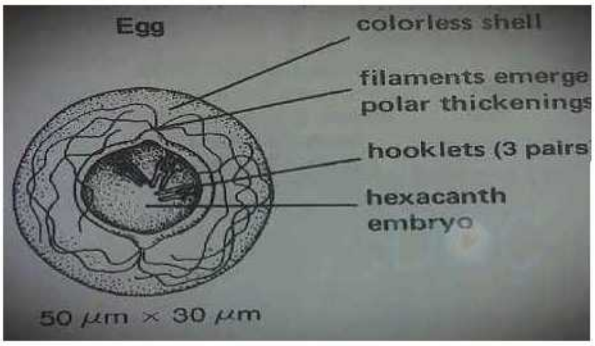

Hooks
Polar thickening
Polar filaments

Egg of H. nana Fig: 2


Egg
colorless shell
filaments emerge
polar thickenings
hooklets (3 pairs
hexacanth
embryo
50 µm × 30 µm

Schematic diagram of Egg of H. nana Fig: 3

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# DIPHYLLOBOTHRIUM LATUM

## Diphyllobothrium latum / Fish tapeworm

00:00:10

- It is the longest cestode

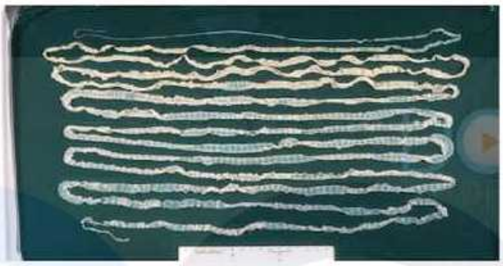

Fig: 1

- Approximately 15metres long
- Definitive host: Man
- Intermediate Hosts are two:
  - 1st is Cyclops – has procercoid larva
  - 2nd is Fish – has plerocercoid larva

Man gets infected by eating fish containing the larva:

Larva reaches intestine

↓

Adults are formed

↓

Fertilization occurs

↓

Females lay eggs

↓

Eggs are passed out in the faeces of man

Eggs – operculated eggs with ciliated larvae inside

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↓

Eggs reach the water

↓

Eggs hatch in water and release ciliated larvae

↓

Motile larvae reach cyclops where they develop further

↓

Cyclops – eaten by fish

Further development of larvae occurs in fish

↓

The fish with larva, when eaten by man, causes infection in man

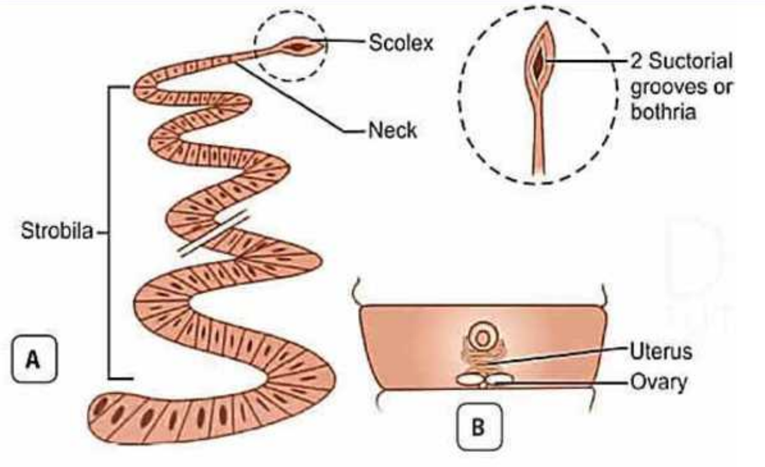
## Clinical features:

- Most infections – asymptomatic
- Some have mild symptoms – abdominal pain and diarrhea
- Classical feature (seen in Scandinavian population) is megaloblastic anemia. This is because, the parasite feeds on vitamin B12 and inhibits ileal absorption of vitamin B12

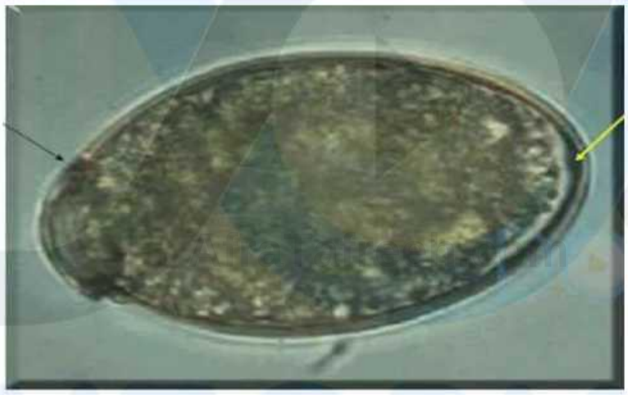
## Diagnosis:

- Faeces examination for eggs and proglottids / segments of the worm

## Treatment: Praziquantel


Scolex
Neck
2 Suctorial grooves or bothria
Strobila
A
Uterus
Ovary
B

Schematic diagram of adult of D. latum Fig: 2



Egg of D. latum Fig: 3

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# TREMATODES - BLOOD FLUKES

## Trematodes/Flukes 00:00:14

- Flat worms – unsegmented body
- Based on the site where adult worms live, they are divided into types.
- Trematodes are hermaphrodites except schistosomes; where male and female sexes are separate

## Blood Flukes/Schistosomes 00:00:38

- DH: Man
- IH: Snail
- Infective form: Cercariae
- Mode of infection: Skin penetration

3 important species:

1. Schistosoma haematobium -lives in vesical venous plexus
2. Schistosoma japonicum
3. Schistosoma mansoni

} live in mesenteric venous plexus

Schistosoma haematobium: causes painless hematuria and squamous cell carcinoma of urinary bladder

- Eggs are excreted in urine
- Eggs of S. japonicum and S.mansoni are excreted in faeces

- Eggs:
  - S. hematobium – in urine, with posterior /terminal spine
  - S. japanicum – in faeces, no spine/rudimentary spine
  - S. mansoni – in faeces, with lateral spine
- Diagnostic stage – eggs
- Infective stage – cercariae larvae

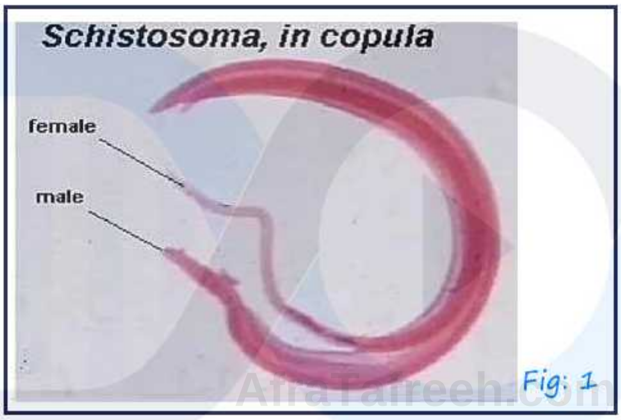
Schistosoma, in copula
female
male

Fig: 1

- Egg of S. hematobium

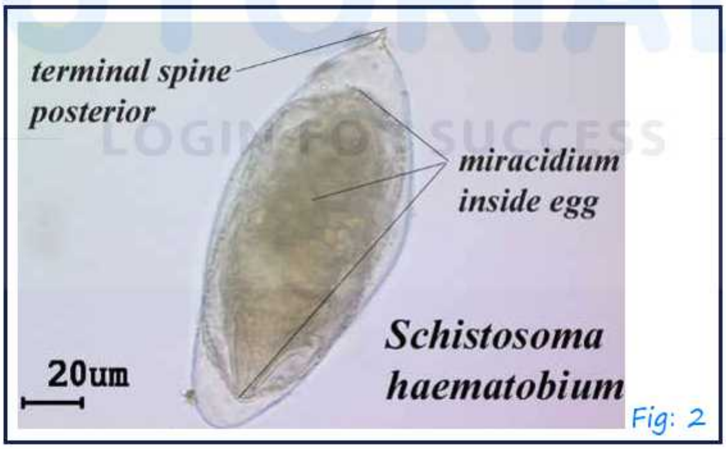
terminal spine
posterior
miracidium
inside egg
Schistosoma
haematobium
20um

Fig: 2

- 3 kinds of eggs:

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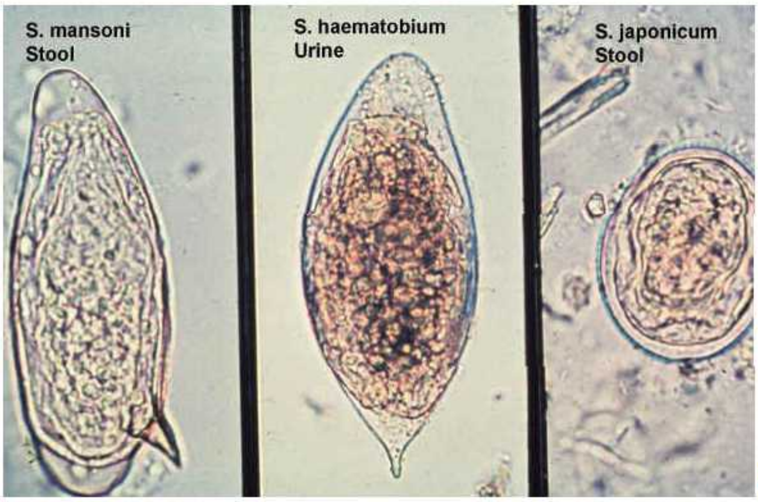
S. mansoni
Stool
S. haematobium
Urine
S. japonicum
Stool

Fig: 3

- S. japanicum and S.mansoni – causes abdominal pain.
- DOC: Praziquantel

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# TREMATODES - LIVER FLUKES

- Fasciola hepatica/Asian liver fluke/Sheep liver fluke
- Clonorchis sinensis / Chinese liver fluke

| Fasciola hepatica | Clonorchis sinensis |
|---|---|
| Disease is caused in sheep and other domestic animals – In Latin America, Africa, Europe | Disease is prevalent in China |
| DH – Man | DH – Man |
| IH – Snail | 2 IH are there:<br>First - Snail<br>Second - Fish |
| Infective form: Metacercariae | Infective form: Metacercaria |
| Mode of infection: Intake of metacercariae encysted in aquatic water plants | Mode of infection: Intake of metacercaria in fish |

## Fasciola hepatica 00:01:46

**Egg is operculated**

- Morphological changes in miracidium are:
  - Sporocyst
  - Radia – 1st gen
  - Radia – 2nd gen
  - Cercaria

**Diagnosis:** faeces examination for eggs

## Clonorchis Sinesis 00:01:46

- Mode of infection: Intake of metacercaria which have encysted in the flesh of fish.

Life cycle of Fasciola hepatica:

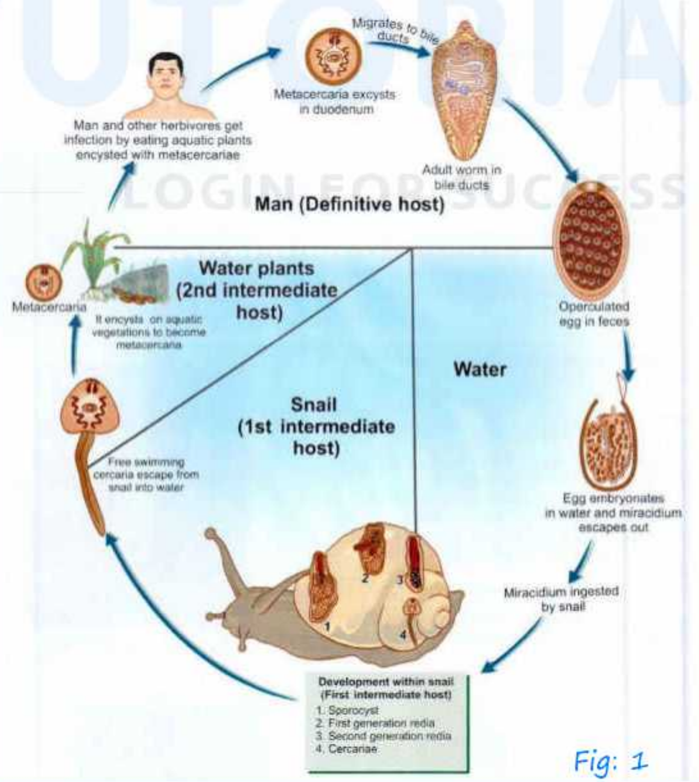
Migrates to bile ducts
Metacercaria excysts in duodenum
Man and other herbivores get infection by eating aquatic plants encysted with metacercariae
Adult worm in bile ducts
Man (Definitive host)
Water plants (2nd intermediate host)
Metacercaria
It encysts on aquatic vegetations to become metacercaria
Operculated egg in feces
Water
Snail (1st intermediate host)
Free swimming cercaria escape from snail into water
Egg embryonates in water and miracidium escapes out
Miracidium ingested by snail
1
2
3
4
Development within snail (First intermediate host)
1. Sporocyst
2. First generation redia
3. Second generation redia
4. Cercariae

Fig: 1

Life cycle of Clonorchis sinensis:

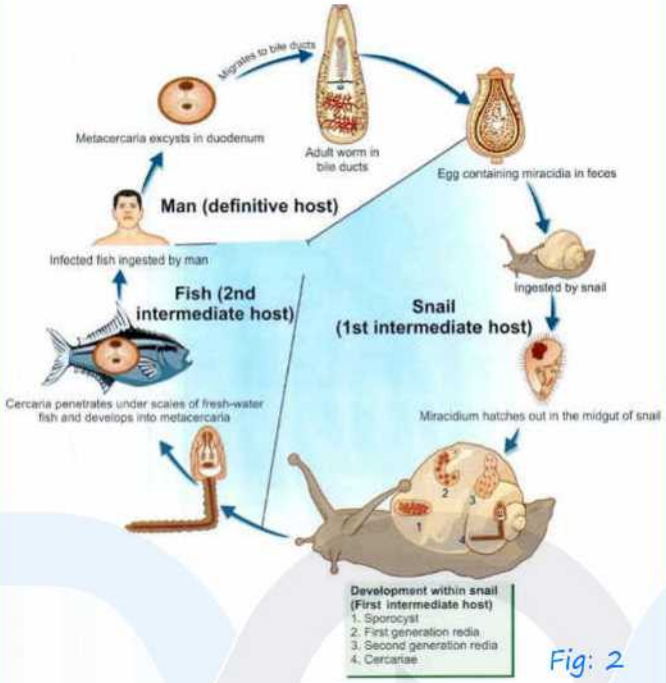
Migrates to bile ducts
Metacercaria excysts in duodenum
Adult worm in bile ducts
Egg containing miracidia in feces
Man (definitive host)
Infected fish ingested by man
Ingested by snail
Fish (2nd intermediate host)
Snail (1st intermediate host)
Cercaria penetrates under scales of fresh-water fish and develops into metacercaria
Miracidium hatches out in the midgut of snail
1
2
3
4
Development within snail (First intermediate host)
1. Sporocyst
2. First generation redia
3. Second generation redia
4. Cercariae

Fig: 2

- This is an example of carcinogenic worm
- It causes cholangiocarcinoma

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## Treatment

- Liver flukes praziquantel

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# TREMATODES - LUNG FLUKE

## Lung fluke 00:00:09

- Paragonimus westermani
- DH: Man
- Intermediate host:
  - First – Snail
  - Second – Crab/Cray fish
- Infective form: - Metacercariae larvae
- Mode of infection: Ingestion of metacercariae present in crab/crayfish
- Life cycle:

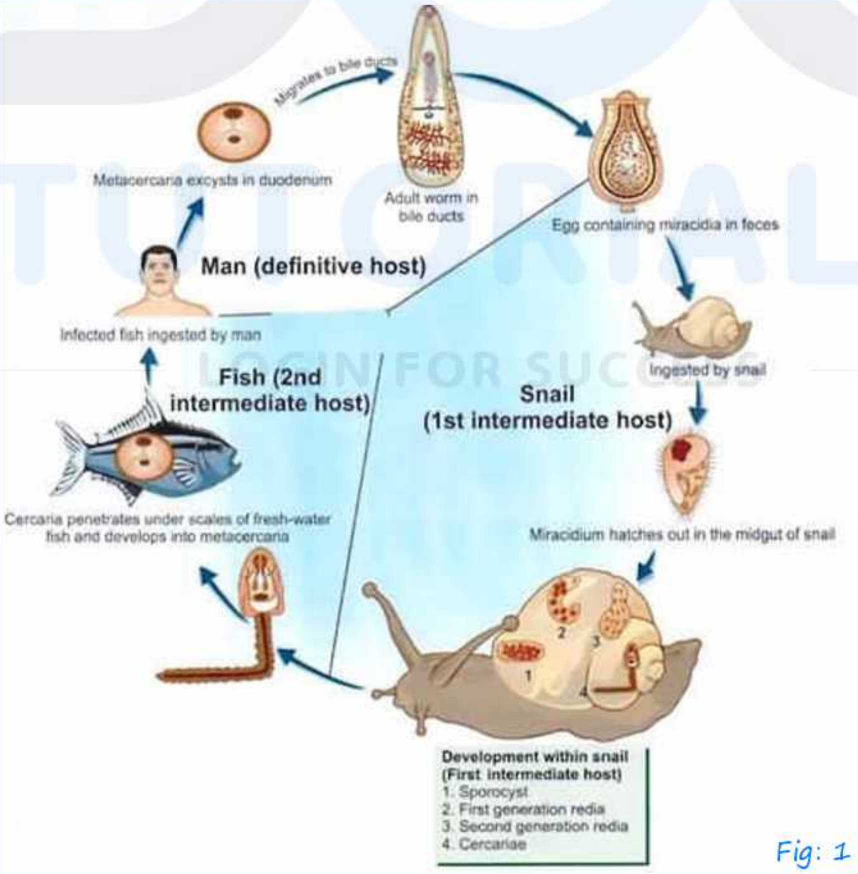
Migrates to bile ducts
Metacercaria excysts in duodenum
Adult worm in bile ducts
Egg containing miracidia in feces
Man (definitive host)
Infected fish ingested by man
Ingested by snail
Fish (2nd intermediate host)
Snail (1st intermediate host)
Cercaria penetrates under scales of fresh-water fish and develops into metacercaria
Miracidium hatches out in the midgut of snail
1
2
3
4
Development within snail (First intermediate host)
1. Sporocyst
2. First generation redia
3. Second generation redia
4. Cercariae

Fig: 1

## Clinical features:

- Endemic hemoptysis
- The disease mimics Tb

## Diagnosis:

- Look for eggs in sputum or faeces

## DOC:

- Praziquantel.

# TREMATODES - INTESTINAL FLUKES

## Intestinal Flukes 00:00:11

- Fasciolopsis buski – Giant Intestinal fluke – largest trematode
  - DH – Man
  - IH – First: snail
    Second: Aquatic plants
  - Mode of infection: Ingestion
  - Infective form: Metacercariae larvae

Life cycle:

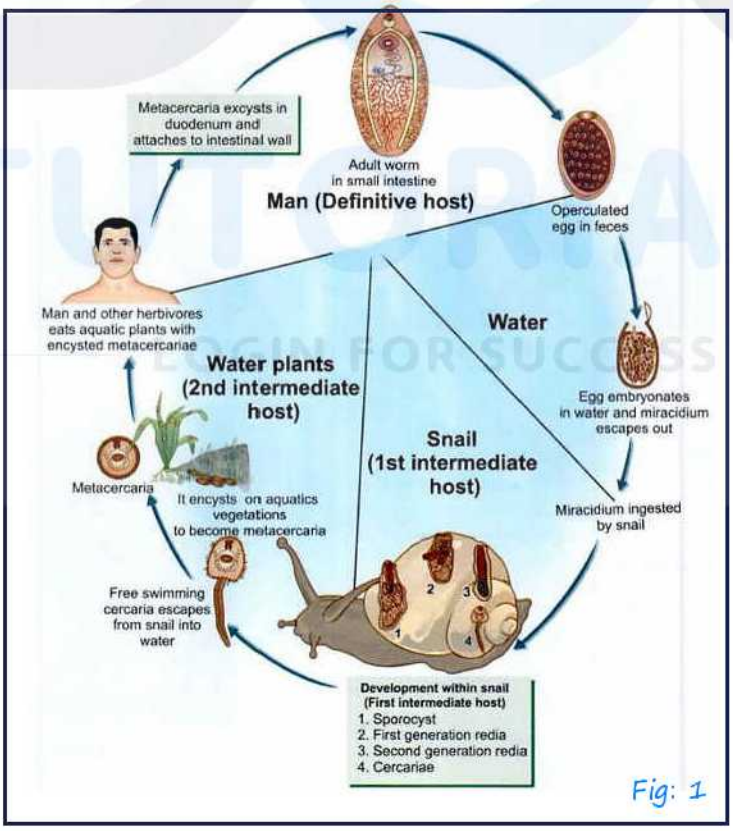
Metacercaria excysts in duodenum and attaches to intestinal wall
Adult worm in small intestine
Man (Definitive host)
Operculated egg in feces
Water
Egg embryonates in water and miracidium escapes out
Miracidium ingested by snail
Man and other herbivores eats aquatic plants with encysted metacercariae
Water plants (2nd intermediate host)
Snail (1st intermediate host)
Metacercaria
It encysts on aquatics vegetations to become metacercaria
Free swimming cercaria escapes from snail into water
1
2
3
4
Development within snail (First intermediate host)
1. Sporocyst
2. First generation redia
3. Second generation redia
4. Cercariae

Fig: 1

- Egg is operculated

### Clinical features:

- Mostly asymptomatic
- Sometimes:
  - Ulcer
  - Abscess
  - Hemorrhage

  All these occur due to mucosal damage by the adult flukes

### Diagnosis:

- Faeces examination for eggs
- DOC: Praziquantel

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## Summary of trematodes 00:04:20

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- For all flukes/trematodes
  - Definitive Host: Man
  - Intermediate Host: Snail
- If 2 intermediate hosts are present:
  - First IH is Snail
- Infective form: Metacercariae larvae
  - Except Schistosomes → cercariae larvae are the infective form
- Mode of infection: Ingestion of infective form except Schistosomes where skin penetration by cercariae is the mode of infection

# MUCORMYCOSIS

## Mucormycosis 00:00:11

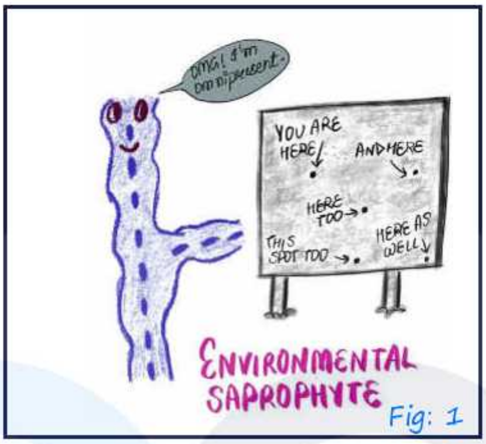
OMG! I'm omnipresent.
YOU ARE HERE
AND HERE
HERE TOO
THIS SPOT TOO
HERE AS WELL
ENVIRONMENTAL SAPROPHYTE

Fig: 1

- Class: Zygomycetes / Phycomycetes (older classification)
- Appearance:
  - Wide (≥ 6-30μm)
  - Thick-walled
  - Ribbon-like
  - Aseptate (Coenocytic)
  - Hyphae branching at right angles

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**Phylum Glomeromycota**

Mucormycotina
↓ Diseases caused are
Mucormycosis

Entomophthoromycotina
↓ Diseases caused are
Entomophthoromycosis
Or
Subcutaneous mucormycosis
- Basidiobolomycosis
- Conidiobolomycosis

## Order Mucorales: organisms included are

- Rhizopus arrhizus (most common)
- Rhizopus microsporus var rhizopodiformis
- Mucor racemosus
- Rhizomucor pusillus
- Apophysomyces species
- Cunnighamella bertholletiae
- Cokeromyces recurvatus
- Saksenaea species
- Syncephalastrum recemosum
- Lichtheimia corymbifera /Absidia corymbifera

## Order entomophthorrales:

- Basidiobolus ranarum
- Conidiobolus coronatus
- Conidiobolus incongruus
- Conidiobolus lamprauges
- Iron is the important growth factor for this fungus
- Sources of Iron:
  - Diabetic ketoacidosis (DKA)
  - Iron therapy
  - Desferrioxamine therapy

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## DKA:

- In DKA, Iron from the serum proteins is dissociated and becomes available for the fungi
- Also, there is decrease in serum $P^H$ in DKA

Decrease serum $P^H$ in DKA

↓

This leads to:

Decrease in phagocytic activity

Decrease in chemotactic effect

Decrease in oxidative burst of phagocytes

## Is black fungus really black?

- This fungus is Angioinvasive
- This fungus has affinity for Elastic Lamina of Small and Large Arteries
- Due to this Angioinvasive property, several manifestations can occur:
  - Thrombosis
  - Hemorrhage
  - Infarction
  - Necrosis

- Neutrophils have special fungicidal activity against zygomycetes

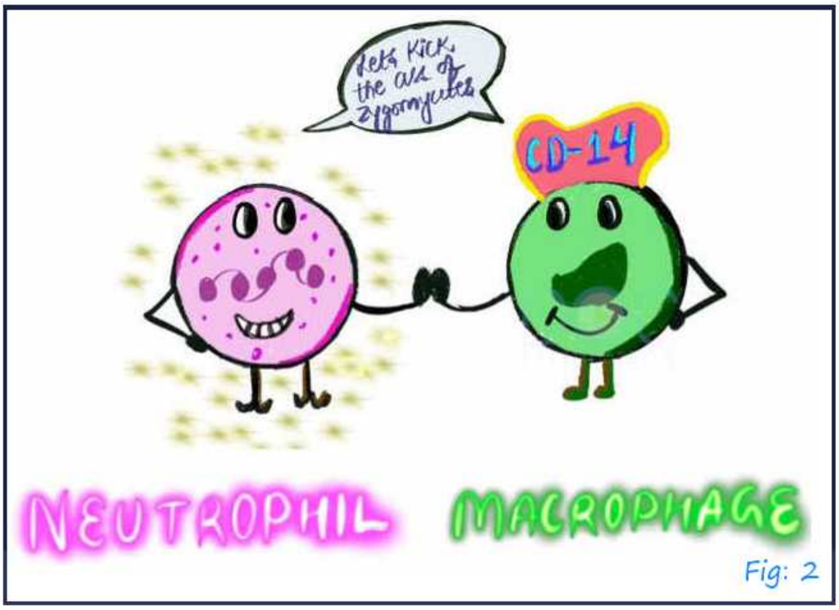
Lets Kick the ass of zygomycetes
CD-14
NEUTROPHIL
MACROPHAGE

Fig: 2

## Risk factors:

- Steroids
- Protein calorie malnutrition
- Leukemia
- Immunodeficiency
- Intravenous drug abusers
- Neutropenia
- Transplant (solid organ, bone marrow)
    - Most commonly encountered organism in Rhizopus arrhizus also known as Rhizopus oryzae

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## Because:

- Angioinvasive
- Growth at/above 37ºc
- Destructive enzymes
- Dormant spores
- Active ketone reductase system
- Hydroxamate siderophores

### Modes of infection:

- Inhalation of spores – most common
- Ingestion of spores
- Inoculation of spores

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## Rhinoorbital cerebral Mucormycosis (ROCM)

00:16:58

---

- It is the most common and fulminating type
- Risk factors:
  - DKA
  - Leukemia and lymphoma
- Order of spread:

  Nasal mucosa

  ↓

  Turbinate bones, PNS, orbit and palate

  ↓

  Brain

  (Frontal lobe neurosis, abscess)

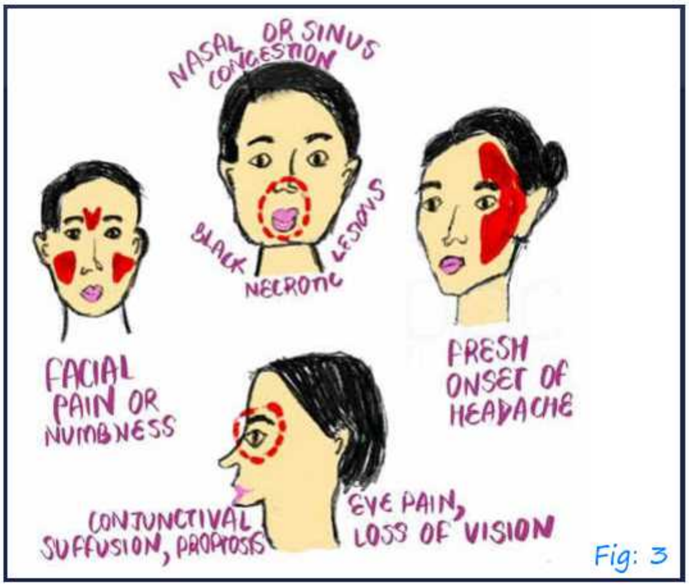
NASAL OR SINUS CONGESTION
BLACK NECROTIC LESIONS
FRESH ONSET OF HEADACHE
FACIAL PAIN OR NUMBNESS
CONJUNCTIVAL SUFFUSION, PROPTOSIS
EYE PAIN, LOSS OF VISION

Fig: 3

- Sudden onset of diplopia in diabetics – think of mucormycosis

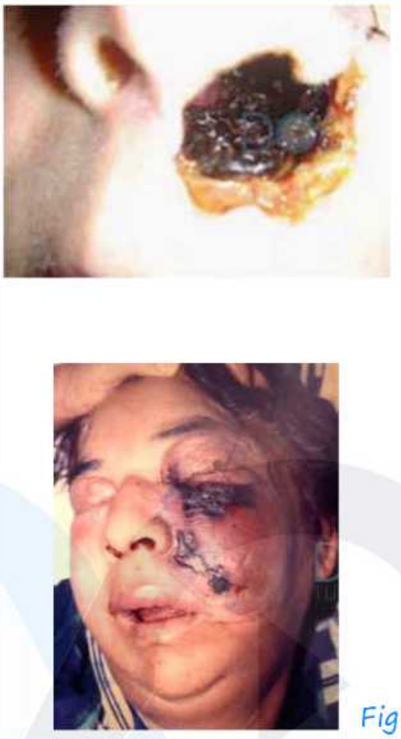

Fig: 4

## Pulmonary zygomycosis 00:19:19

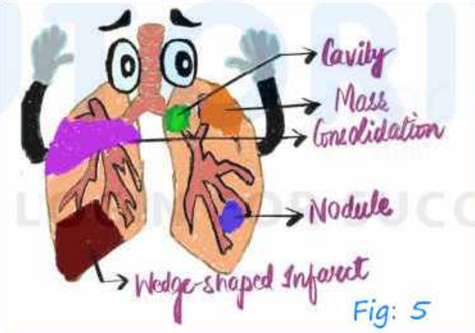
Cavity
Mass
Consolidation
Nodule
Wedge-shaped Infarct

Fig: 5

- It is the second most type of zygomycosis
- Patient presents with cough, chest pain, dyspnea, fever
- Very rarely – fungal ball

- Hypersensitivity pneumonitis due to Rhizopus has been reported in Scandinavian sawmill workers (wood trimmer's disease) and in farm workers

## Gastrointestinal Mucormycosis 00:20:55

- Most common site: stomach
- 7% of all cases
- Risk factors: extreme malnutrition
- It is acquired by ingestion of food containing spores

  Other sites of GIT involved:
  - Colon – seen in abdominal surgery
  - Ileum – contaminated ileostomy
  - esophagus
- Signs and symptom:
  - Abdominal pain
  - Diarrhea
  - Hematemesis
  - Malena

## Isolated renal mucormycosis 00:22:53

- Pathophysiology – angioinvasion
- There is:
  - Flank pain
  - Fever
  - Pyuria
  - Hematuria
- Case fatality rate: 65%

## Cutaneous Mucormycosis 00:23:48

- It can be primary or disseminated
- Primary cutaneous mucormycosis occurs in
  - Superficial ulcers in diabetics
  - Gangrenous cellulitis in trauma patients
  - Contaminated elasticized wound dressings

### Clinical features:

- Pustules
- Vesicles
- Necrotic wounds

### In early stages: lesions resemble Ecthyma Gangrenosum

- There may be appearance of "Hairy pus"
- Lesions are painful
- Lesions gradually become nodular ecchymotic areas
- Necrotizing fasciitis can occur in cutaneous and subcutaneous mucormycosis. It has high CFR
  - Lichtheimia corymbifera and
  - Saksenaea
- These are most important organisms responsible for cutaneous mucormycosis in diabetic patients

Fig: 6

## Diagnosis 00:26:24

### Sample collection:

- ROCM:
  - Scraping/exudate from nares
  - Hard palatal lesions, sinus material, biopsy from extracted tooth socket area
  - Endoscopic collection of debrided tissue/biopsy

    Dry nasal swabs – are not useful
- Pulmonary mucormycosis:
  - Sputum
  - Bronchial brush washing/BAL
  - Lung biopsy
  - Fluoroscope – guided transthoracic needle aspiration or open lung biopsy

    Saliva saline wash – are not recommended
- GIT Mucormycosis:
  - Endoscopic biopsy of lesion
  - Faeces – not recommended
- Renal mucormycosis:
  - Biopsy of the lesions
  - Urine – not recommended
- Cutaneous mucormycosis:
  - Aspirate from undrained abscess (with sterile needle and syringe)
  - Pus expressed from abscess opened with scalpel

  - Transport to lab in sterile container/syringe a needle
  - Tissue from both Centre and edge of lesion should be collected

    Swab or materials from open wounds, dry swabs are not recommended

- The tissue should not be homogenized, otherwise hyphae break and correct morphology cannot be appreciated

## Microscopic examination:

- KOH Mount
- PAS stain – best
- HnE stain
- GMS stain
- Gram stain:
  - All fungi are Gram positive
  - Filamentous fungi take up faint blue color (missed sometimes)
  - Mucor on GMS stain:

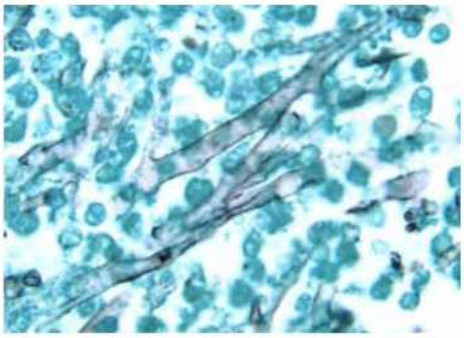

Fig: 7

  - KOH mount

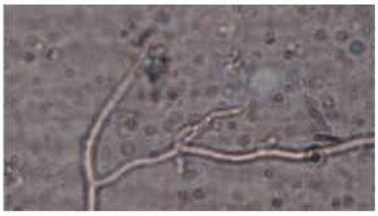

Fig: 8

- Rhizopus in tissue = HnE

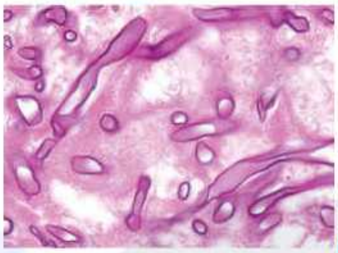

Fig: 9

## Culture:

- Cotton candy growth
- Salt and pepper appearance of Rhizopus

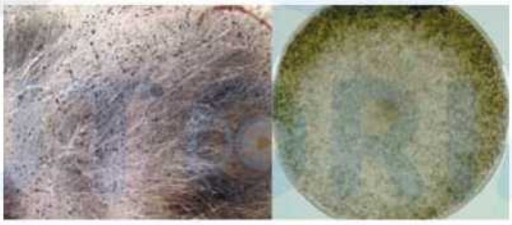

Fig: 10

- Lid lifters (because of aerial growth)

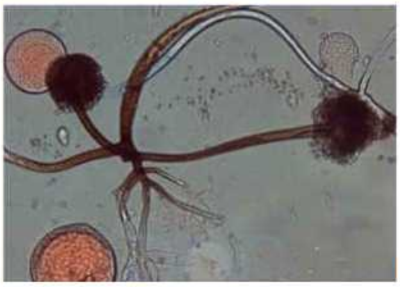

Fig: 11

- Rhizoids – root like structures aseptate hyphae

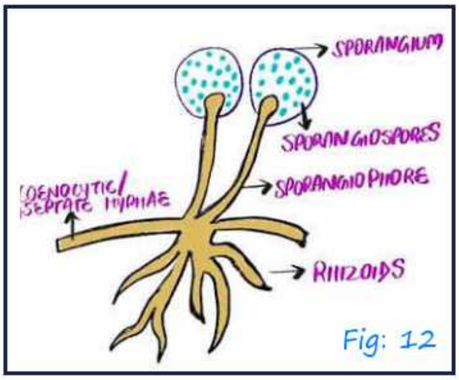
SPORANGIUM
SPORANGIOSPORES
SPORANGIOPHORE
COENOCYTIC/ ASEPTATE HYPHAE
RHIZOIDS

Fig: 12

- Sporangiophore: stalk/stem with sporangium on the stem
- Sporangiophores: Asexual spores
- Mucor, LPCB mount

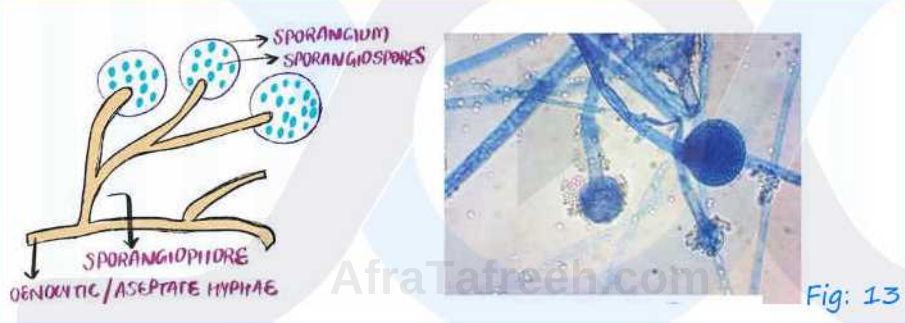
SPORANGIUM
SPORANGIOSPORES
SPORANGIOPHORE
COENOCYTIC/ASEPTATE HYPHAE

Fig: 13

- No rhizoids
- They also produce sexual spores – zygospores and oospores

**Treatment:**

- Mainstay is surgical debridement
- Antifungals:
  - Amphotericin β
  - Posaconazole
  - Isavuconazole
- Cytokines: IFN – α, GM-CSF